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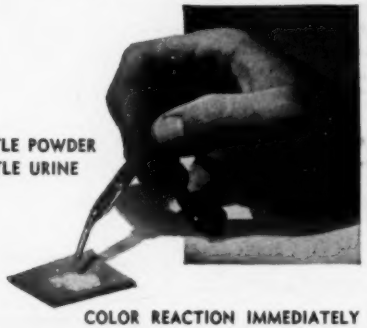

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CONTENTS

PAGE

Effect of Experimental Shock Induced by Clostridium Perfringens Toxin on the Kidneys of Dogs. M. Berg, M.D., Ph.D.; S. A. Levinson, M.D., and K. J. Wang, M.S., Chicago 137

Endophlebohypertrophy and Phlebosclerosis: I. The Popliteal Vein. Maurice Lev, M.D., and Otto Saphir, M.D., Chicago 154

Acute Bacterial Endocarditis Due to Pseudomonas Aeruginosa: Report of a Case. Frederick C. Coller, M.D., and Dallas J. Dyer, M.D., St. Louis 179

The Sympathetic Ganglions of Hypertensive Patients. R. C. Truex, Ph.D., Philadelphia 186

The Postnatal Structural Changes in the Intrapulmonary Arteries and Arterioles. W. Harold Civin, M.D., and Jesse E. Edwards, M.D., Rochester, Minn. 192

Quantitative Exfoliative Cytology: Differential Counting of Cervical Smears Stained for Glycogen in Cases of Pregnant and Nonpregnant Women. Alvan G. Foraker, M.D., and Darnell L. Brawner, M.D., Atlanta, Ga. 201

Aberrant Pyloric Glands in Regional Ileitis. Amour Fiscus Liber, M.D., Yonkers, N. Y. 205

Variants of the Platelet Thrombosis Syndrome and Their Relationship to Disseminated Lupus. Paul M. Beigelman, M.D., Los Angeles 213

Lipids of Normal and Atherosclerotic Aortas: A Chemical Study. Robert C. Buck, M.D., M.Sc., and Roger J. Rossiter, M.A., M.Sc., B.M., B.Ch., D.Phil., London, Ont., Canada 224

CASE REPORTS:

Melanoma of Brain and Gallbladder. E. E. Pautler, M.D., Richmond, Va., and E. M. Gallavan, M.D., Pueblo, Colo. 238

NOTES AND NEWS 246

BOOKS RECEIVED 247

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EFFECT OF EXPERIMENTAL SHOCK INDUCED BY CLOSTRIDIUM PERFRINGENS TOXIN ON THE KIDNEYS OF DOGS

M. BERG, M.D., Ph.D.
S. A. LEVINSON, M.D.
AND
K. J. WANG, M.S.
CHICAGO

THE EXPERIMENTAL study of traumatic shock and the so-called "lower nephron syndrome" has followed several classic approaches, such as the application of crushing injuries to an extremity, repeated hemorrhages, burns, the injection of extracts of traumatized muscles and the injection of bacterial toxins. Clostridium perfringens has been observed to occur in war wounds, and it, too, has been associated with a shock syndrome.[1] The physiological and pathological actions of various fractions of Clostridium toxin administered intravenously and intramuscularly to induce experimental shock in dogs [2] has since been reported. Since the action of the toxins produced by clostridia depend on the amount used and on the route and the rate of injection, considerable attention has been given to these factors. Studies of the effects of the purified alpha toxin injected intramuscularly revealed little edema around the site of injection, while studies of the purified Cl. welchii hyaluronidase similarly revealed little effect on intramuscular injection. The edema formation following intramuscular or subcutaneous injection of the unpurified Cl. welchii toxin filtrate appears to be due to other toxins present or to a synergistic effect of the combination of toxins. Intravenous injections of Cl. welchii toxin resulted in intravascular hemolysis followed by shock. Clostridium novyi (or oedematiens) toxin when administered in purified form intravenously,[3] on the other hand, produced a gradual picture of shock and death, and when administered intramuscularly, produced massive local edema. These observations have prompted a further investigation of the problem with particular emphasis on the physiological and pathological alterations of the kidneys as related to the controlled development of traumatic shock induced

From the Hospital Laboratory, Research and Educational Hospitals, the Departments of Pathology, Medicine and Clinical Sciences, University of Illinois College of Medicine.

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by intramuscular injections of Cl. perfringens (welchii) toxin. A variable result was observed in preliminary experiments employing small doses of the toxin; large doses were administered to insure uniformity and rapidity of action. While objections have been raised to the intramuscular use of large doses of perfringens toxin, it has, in addition to the advantages of uniformity and rapidity of action, facility in modifying its action, which should yield controlled and significant observations.

FIRST EXPERIMENT

Experimental Procedure.—The dogs were anesthetized with pentobarbital sodium, given intravenously (30 mg. per kilogram of body weight). The M. L. D. of Cl. perfringens toxin [4] was 8 ml. per kilogram of body weight, which, in a series of preliminary experiments on eight dogs, was found to be the amount which when injected intramuscularly into the midportion of the hind-leg would kill the animal within 5½ to six hours. This dose was employed in all the experiments. As a control, injections of equal amounts of culture medium alone, filtered and prepared in the same manner as that containing the toxin, were found to be without lethal effect. The following determinations were carried out at regular intervals on the experimental and the control animals by the methods cited: mean arterial blood pressure (readings made by the direct method from the femoral artery); hematocrit levels (Wintrobe method), leg volume,[5] urine volume and constituents, blood chlorides,[6] blood urea nitrogen,[7] serum potassium,[8] serum sodium [9] and alkaline phosphatase.[10]

Results.—A series of eight control animals were anesthetized and observed in the same manner as the animals given injections of the perfringens toxin; no significant alterations were observed in the hematocrit readings, blood pressure observations, blood chemical determinations and urine volume. In a series of 15 animals given the injection of perfringens toxin, the blood pressure readings revealed a gradual reduction of pressure over a five hour period. With the control blood pressure expressed as 100 per cent, the hourly average pressures were found to be 92, 87, 82 and 76 per cent, respectively (fig. 1). On the other hand, the urinary output for this group showed a statistically significant decline in the first hour after injection of the toxin. The urinary volume during the control period was 6 ± 1 ml., and during the first hour after injection of toxin it decreased to 2 ± 0.67 ml. Subsequently, the urine output continued to diminish, and after four hours no urine was excreted. In this the intoxicated animals were in sharp contrast to the control animals, which showed no decline in urinary volume throughout the experiment. In the group receiving the toxin a few

4. The Cl. perfringens toxin used was furnished by Eli Lilly & Company, Indianapolis.

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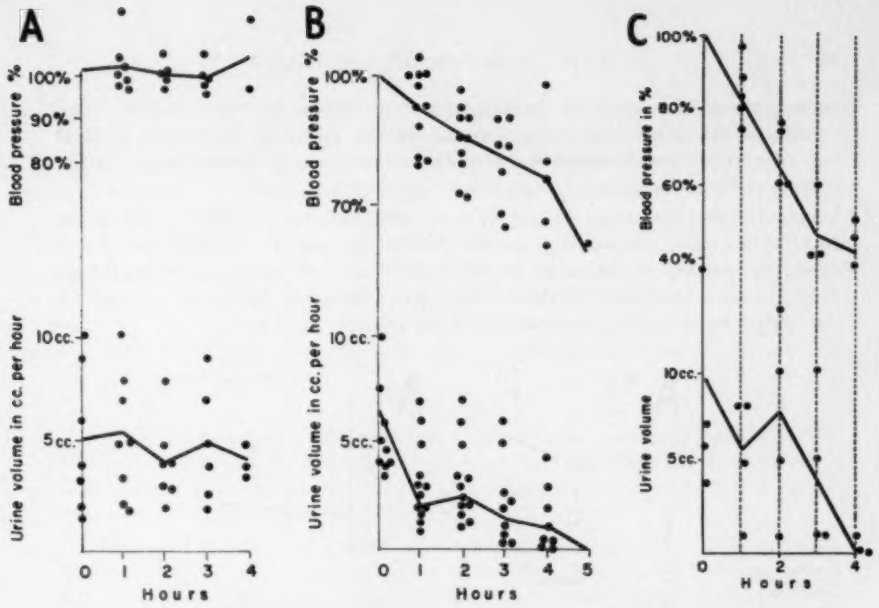

Fig. 1.—The hourly blood pressure determinations (expressed as per cent of the initial reading) and the hourly urinary output of (*A*) controls, (*B*) animals that had received in a hind-leg an intramuscular injection of Cl. perfringens toxin and (*C*) animals that had a plaster cast applied to the leg immediately after the injection of the toxin.

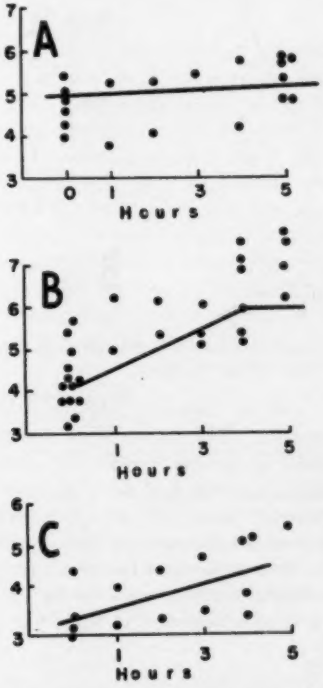

Fig. 2.—The hematocrit determinations of (*A*) controls, (*B*) the group that had received an injection of toxin and (*C*) the group with a cast applied immediately after the injection.

animals showed no significant drop in volume of urine during the first hour. The hematocrit determinations revealed in the animals receiving the toxin a gradual but significant hemoconcentration reaching a maximum in four hours. In the control group no hemoconcentration was observed (fig. 2).

Hourly determinations of leg volume following the injection of the toxin revealed no significant swelling for two hours, after which considerable swelling occurred, reaching its maximum in the third hour. The leg volume [5] determination showed a maximum increase which varied between 1.6 and 3 per cent of the body weight, with an average of 2 per cent.

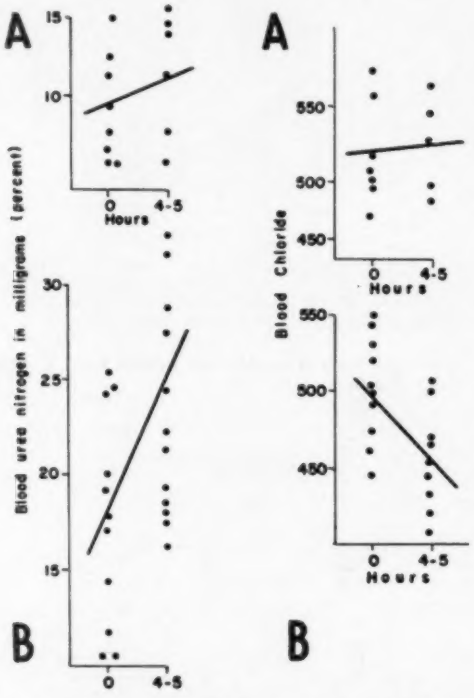

Fig. 3.—The blood urea nitrogen and blood chloride determinations of (*A*) controls and (*B*) the animals that had received an injection of toxin.

The urinary findings were as follows: The urine of the control animals showed no microscopic changes. The hourly urine samples of the animals that received the toxin contained a variable amount of white and red blood cells, albumin, a pinkish amorphous material and, most characteristically, an increasing number of well formed granular casts. The terminal urine samples were usually dark reddish brown and contained numerous casts, sediment and cells. The control animals showed no decline in excretion of urinary chlorides for the first three hours; however, a decline was observed during the fourth hour. In 7 of 10 toxin-treated animals a decline occurred in excretion of chlorides during the

first hour; all showed a significant decline in the second hour, with a progressive decrease in urinary chlorides in the succeeding hours. Serial determinations of urinary p_H and spectroscopic studies of urine and blood were not carried out.

Studies of the blood urea nitrogen of the toxin-injured animals revealed a statistically significant increase after four hours (fig. 3); the control period average was 17.8 ± 1.84 mg. per 100 ml., and after four hours it was 25 ± 3.16 mg. per 100 ml. The blood chlorides showed a small but consistent drop over the same period. The values before and four hours after injection of toxin were as follows: sodium, 148 ± 4 mEq. and 140 ± 8 mEq. per liter; potassium, 4.7 ± 0.1 mEq. and 5.0 ± mEq. per liter; alkaline phosphatase, 2.1 ± 1.0 and 2.7 ± 1.3 Bodansky units.

SECOND EXPERIMENT

In order to evaluate and to correlate the behavior of the kidneys with the chemical and physiological changes noted, a series of simultaneous studies (histochemical and histological) of the glomerular and tubular functions and of the structural alterations were performed.

Experimental Procedure.—The animals were killed at hourly and half-hourly intervals after the injection of the toxin. Six minutes before the animals were killed, they were given phenol red[11] (1 ml. per kilogram) and sodium ferrocyanide[12] (5 ml. per kilogram) intravenously. It had been demonstrated previously that the phenol red is excreted chiefly by the proximal convoluted tubules[11] and the ferrocyanide by the glomeruli.[12] The kidneys were removed quickly, and sections of the kidney, as well as sections from other organs, were placed in isopentane, rapidly frozen to — 145 C. and dehydrated after the method of Gersh and Bensley, an apparatus designed by one of us (Wang)[13] being employed in this operation. Other sections for special studies were fixed in formaldehyde solution U. S. P. diluted 1:10, Regaud's fluid, alcohol and acetone for appropriate fixation.

Results.—Preliminary experiments were carried out to study the anatomic changes resulting from injections of lethal doses of perfringens toxin. All the experimental animals showed distinct edema of the leg that received the injection. There was evidence grossly and microscopically of localized hemorrhages involving the muscles and subcutaneous tissues; however, there was no evidence of muscle necrosis. It was noted that there was severe congestion of the intestinal tract beginning sharply at the first portion of the duodenum and diminishing gradually to disappear in the ileum. None of the other organs showed any remarkable changes grossly except the kidneys, which were swollen and purplish. Microscopic examination of all the organs revealed capillary congestion, more marked in the lungs, and present to a lesser extent in the liver and the intestinal tract. The congestion of the kidneys was very pronounced; the proximal convoluted tubules showed moderate to maximal cloudy swelling and necrosis, and the distal convoluted tubules were partly filled with a pink amorphous material; the cytoplasm was coarsely granular, and few nuclei were seen. In the descending limb of Henle's loop the cell outlines were indistinct and the nuclei pyknotic. In the

11. Gersh, I.: Tubular Elimination of Phenol Red in Rabbit Kidney, Am. J. Physiol. **108**: 355, 1934.

12. Gersh, I., and Steiglitz, E. J.: Histochemical Studies on Mammalian Kidney: Glomerular Elimination of Ferrocyanide in Rabbit, and Some Related Problems, Anat. Rec. **58**:349, 1934.

13. Wang, K. J., and Grossman, M. I.: A Simplified Vacuum Dehydration Technique for the Preparation of Sections by Freezing-Drying, J. Lab. & Clin. Med. **34**:292, 1949.

ascending limb the cells were swollen, the nuclei prominent, and there was cytoplasmic vacuolation, with amorphous material in the lumen. These findings were compatible with those seen in animals dying of shock.

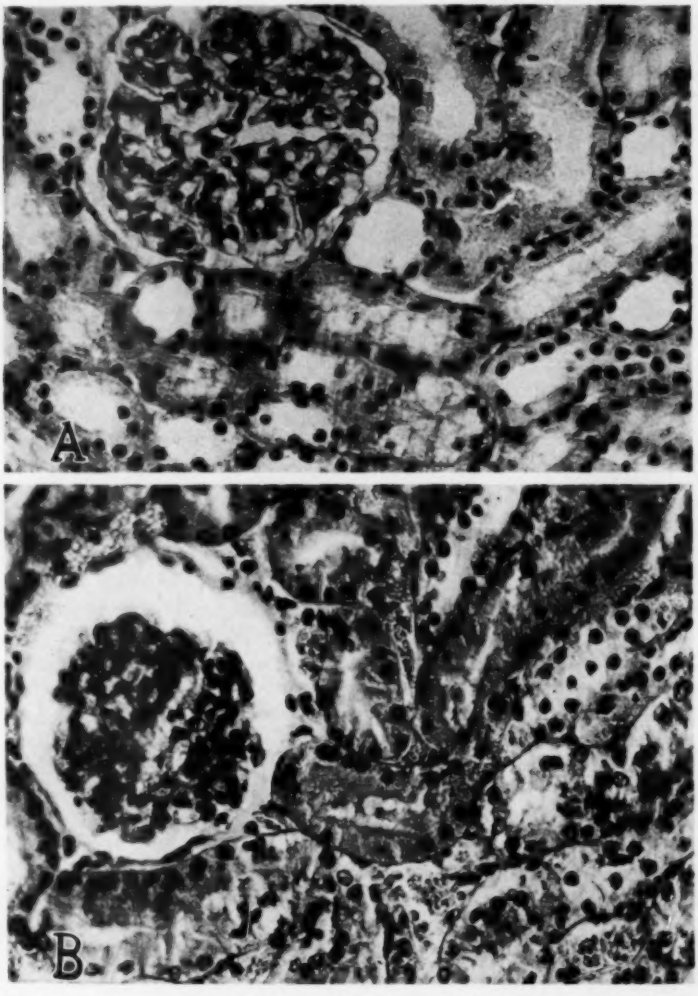

Fig. 4.—*A*, kidney removed 3½ hours after injection of toxin, showing swelling of the glomerulus and of the epithelium of the proximal convoluted tubules.

B, kidney removed five hours after injection of toxin, showing marked capillary congestion, a shrunken glomerular tuft and severe tubular degeneration (hematoxylin and eosin stain).

Serial observations of the kidneys revealed the following:

One to 1½ hours after injection: No remarkable changes were seen.

Two to 2½ hours after injection: Approximately 50 per cent of the glomerular tufts were devoid of red blood cells. The tubular epithelium appeared normal, with some pinkish globules in the lumen of some of the proximal convoluted tubules.

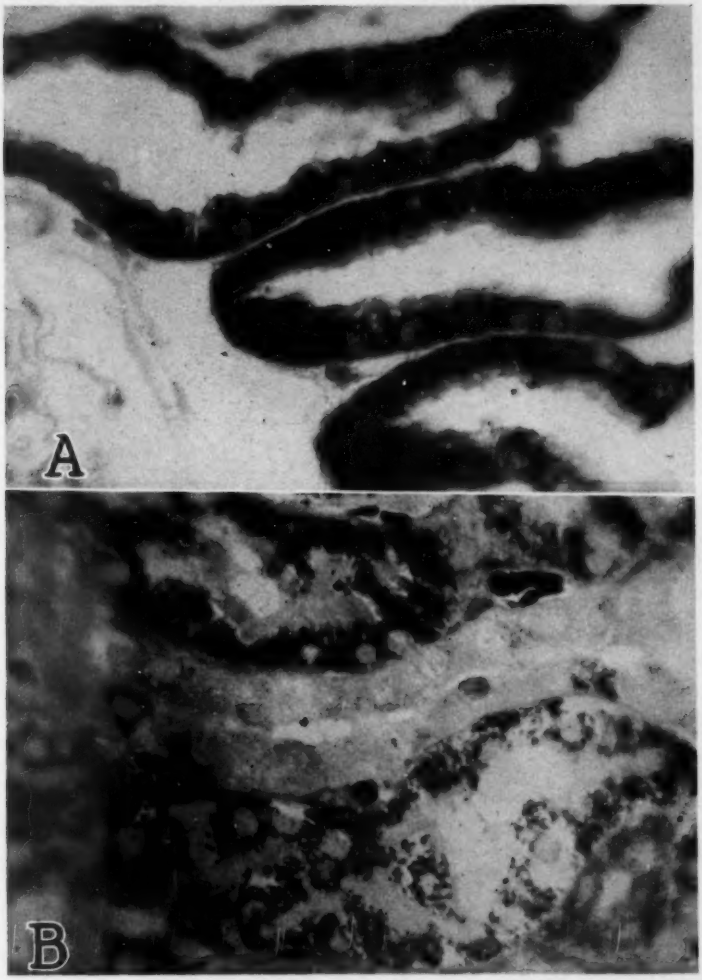

Fig. 5.—*A*, kidney removed three hours after injection of toxin, showing normal-appearing mitochondria in the proximal convoluted tubules. Magnification, × 800.

B, kidney removed five hours after injection of toxin, revealing varying degrees of fragmentation and condensation of the mitochondria of the proximal convoluted tubules. Magnification, × 800.

In scattered tubules there was cytoplasmic vacuolation, and a pinkish-stained material was noted in the lumen in Henle's loop. The other histological observations

were within the normal range, and the mitochondria of this group were unchanged (fig. 5 *A*). The phenol red appeared to be in normal concentration in the proximal

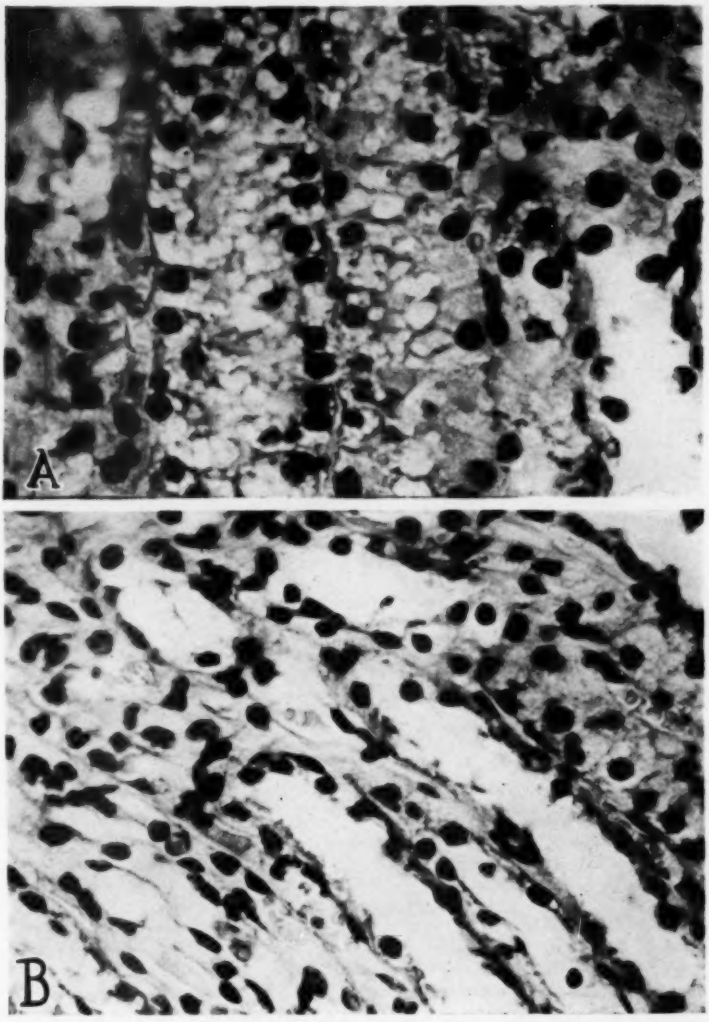

Fig. 6.—*A*, kidney removed 4½ hours after injection of toxin, showing extensive vacuolation and disintegration in the distal portion of the proximal convoluted tubules (hematoxylin and eosin stain). Magnification, × 400.

B, kidney of another animal removed 4½ hours after injection of toxin, showing swelling of the cells of the ascending limb of Henle's loop (hematoxylin and eosin stain). Magnification, × 400.

convoluted tubules, and the sodium ferrocyanide was present in all the structures of the nephron but was more concentrated in Henle's loop.

Three, 3½ to four hours after injection: Increasing numbers of glomeruli were swollen, and the capillaries and arterioles were free of blood (fig. 4 *A*). Some of the proximal convoluted tubules were unchanged, while others showed granular cytoplasmic swelling with nuclear fragmentation and pyknosis; some of the nuclear fragments appeared free in the lumen. Considerable variation of the mitochondrial alterations in the proximal convoluted tubules were observed, with many of the cells showing fragmentation and condensation of the mitochondria (fig. 5 *B*). In some sections only a few epithelial cells were involved; in others the involvement was more widespread. In this group the ferrocyanide was seen to be either absent or markedly diminished in amount in many of the glomeruli. The swollen glomeruli

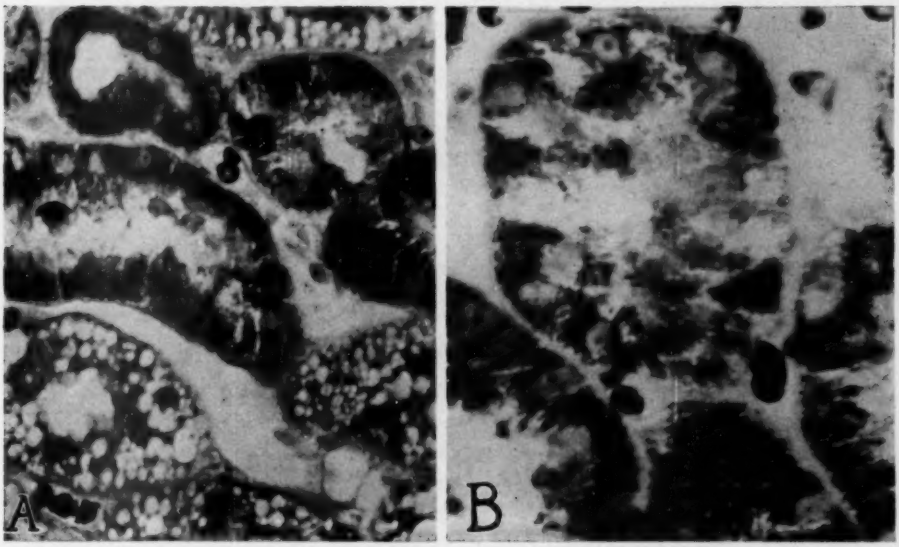

Fig. 7.—*A*, kidney removed five hours after injection of toxin, showing areas of more severe and widespread mitochondrial alterations in the proximal convoluted tubules. Magnification, × 800.

B, higher magnification of *A*, showing variations in the severity of the mitochondrial changes in the cells of a proximal convoluted tubule. Magnification, × 1,000.

showed little or no ferrocyanide. Variable amounts of phenol red were seen in the proximal convoluted tubules.

Four and one-half to five hours after injection: The histological changes were quite striking. In a number of specimens obtained five hours after injection the glomeruli appeared shrunken but presented appreciable congestion of the glomerular capillaries as well as general congestion of the kidneys (fig. 4 *B*). In the proximal convoluted tubules the changes varied from minimal to severe vacuolation, extensive disintegration and necrosis. In some of the specimens extensive vacuolation and disintegration were observed in the distal portions of the proximal convoluted tubules (fig. 6 *A*). In some specimens swelling of the cells of the ascending limb of Henle's loop was seen (fig. 6 *B*).

In this last group the mitochondrial changes presented a variable picture. For example, in a cross section of a proximal convoluted tubule, while several cells presented a normal appearance, the remainder showed fragmentation, with condensation of the particles (fig. 7 *A*). In other sections there were widespread and severe alterations, such as a pronounced reduction of the number of mitochrondria and an increase in size of the fragments to almost complete absence of mitochondria (figs. 7 and 8). Even where the destruction was severe and widespread, a few small areas were observed in which the mitochondria appeared fairly normal.

The ferrocyanide was observed in large amounts in the congested glomeruli as well as in the lumens of the proximal convoluted tubules, in Henle's loop and in the dilated capillaries. On the other hand, no phenol red was visible in the proximal convoluted tubules, with the rare exception of a few islets of secreting cells.

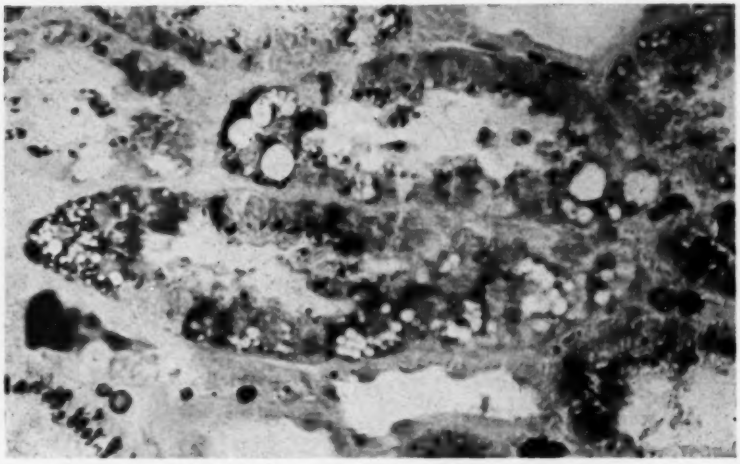

Fig. 8.—Another area of the kidney seen in figure 7, showing the almost complete destruction of the mitochondria of the proximal convoluted tubules. Magnification, × 800.

THIRD EXPERIMENT

The effects of the application of a cast in minimizing the local edema and on the dynamic changes observed following the experimental crushing of an extremity [14] and following the injection of Clostridium toxin [15] have been previously reported. Therefore a series of observations were carried out in order to study the effect on the dynamic changes (circulatory, chemical) of a plaster cast applied to the extremity

14. Duncan, G. W., and Blalock, A.: The Uniform Production of Experimental Shock by Crush Injury: Possible Relationship to Clinical Crush Syndrome, Ann. Surg. **115**:684, 1942. Eggleton, M. G.; Richardson, K. C.; Schild, H. O., and Winton, R. R.: Renal Damage Due to Crush Injury and Ischemia of Limbs of Anaesthetized Dogs, Quart. J. Exper. Physiol. **32**:89, 1943-1944. Swingle, W. W.; Remington, J. W.; Kleinberg, W.; Drill, V. A., and Eversole, W. J.: Experimental Study of Tourniquet as Method for Inducing Circulatory Failure in Dog, Am. J. Physiol. **138**:156, 1942.

15. Zamecnik and others.[2b] Kety and others.[5]

in which toxin had been injected and its influence on the cellular function and structure of the kidney. A second series of experiments were performed to study the effect of increasing the amount of toxin in the blood stream by means of massage at the site of injection.

Experimental Procedure.—In a series of four animals prepared in the manner previously described, the hindleg that had received the injection of toxin was encased in a plaster cast immediately after the injection. In a second series of three animals, the toxin-treated hindleg was massaged after the injection in order to increase the rate of absorption of the toxin.

Results.—In the first group of animals the hourly blood pressure readings revealed a decline over a five hour period. With the control pressures expressed as 100 per cent, the hourly average pressures were found to be 85, 67, 46 and 21 per cent, respectively (fig. 1).

The urinary output of the first group did not show a significant diminution until four hours after injection. During the control period it was 9.4 ± 3.8 ml. and at four hours it was 0.3 ± 0.3 ml., at which time most of the animals failed to secrete any urine.

There was a slight increase in the hematocrit levels, the control values being 3.7 ± 0.38 cm. and the values at four hours 4.5 ± 0.5 cm. These results were compared with results of similar studies of the group of animals which received the toxin alone, in figure 2 *B* and *C*.

Microscopic studies of the hourly urine specimens in the plaster cast group of animals did not reveal any remarkable changes until two hours after the injection, at which time there was noted an increased number of red and white cells. After three hours the presence of granular casts was first noted, and the number of cellular constituents was notably increased. The abnormal cellular constituents of the urine appeared earlier and in larger numbers in the animals that did not have a plaster cast. The excretion of urinary chlorides remained unaltered in the animals with the cast.

In the studies of the blood chemistry of the animals with the plaster cast there was observed a tendency for the blood urea nitrogen to rise and for the blood chlorides to fall after the injection, but the variations in amounts were small. The serum sodium and potassium and the alkaline phosphatase were unaltered.

The microscopic studies of the kidneys of this group which were removed four and five hours after the injection revealed that the glomeruli, for the most part, were normal in size and appearance, with the exception that the glomerular capillaries were devoid of blood cells; only in one five hour specimen were some of the glomeruli congested. The cells of the proximal convoluted tubules showed considerable variation. Many of the tubules appeared normal. Other tubules showed alterations varying from cloudy swelling to necrosis. The areas showing necrosis were small and infrequent. A number of tubules presented vacuolation of the cytoplasm. There was considerable amorphous debris, unformed and formed, in the distal convoluted tubules.

The mitochondrial changes in the proximal convoluted tubules varied, many of the cells appearing fairly normal and others showing more fragmentation and condensation of the mitochondria.

The sodium ferrocyanide was present in normal amounts in many of the glomeruli and in the lumens of the proximal convoluted tubules.

The phenol red either appeared very faint or, for the most part, was not visible in the cells of the proximal convoluted tubules.

In the group of animals that received the toxin and massage, the hourly blood pressure readings revealed a slow decline. The readings were 100, 82, 82, 80 and 79 per cent, respectively.

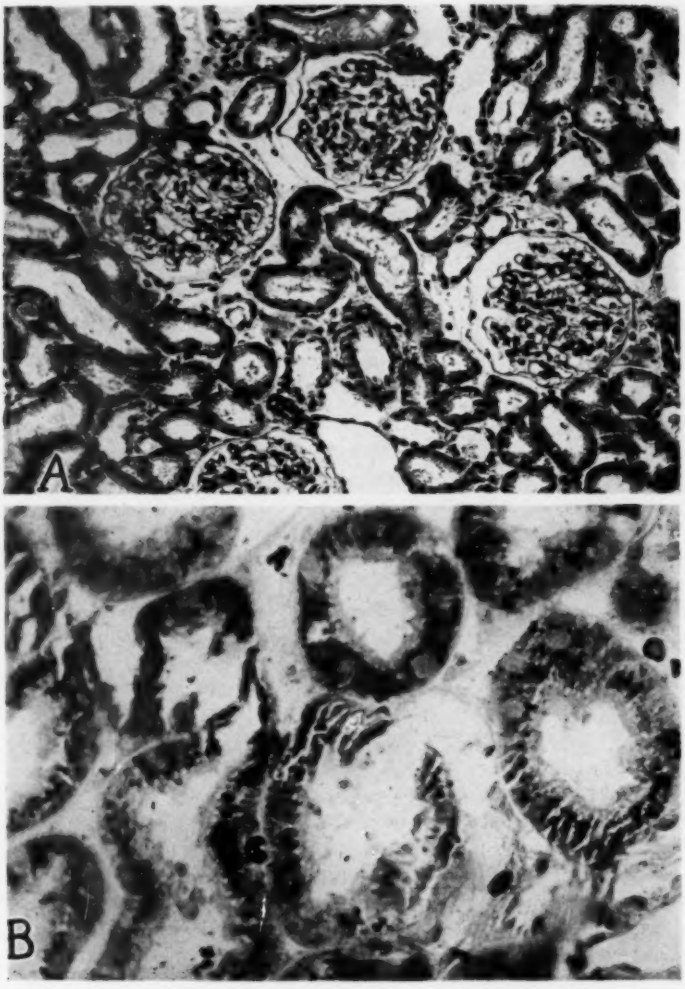

Fig. 9.—*A*, kidney removed four hours after injection of toxin and massage of the site of injection. Note glomerular swelling, presence of hemoglobin in Bowman's space and minimal swelling of the proximal convoluted tubules (hematoxylin and eosin stain). Manification, × 200. *B*, higher magnification of *A* (× 800). Note extensive and severe mitochondrial alteration, evidenced by the disappearance and clumping together of the mitochondria.

The urinary output showed a significant drop one hour after injection, and there was no urine four hours after injection. In this group gross blood was seen in the

urines two hours after the injection. Microscopically, in the first hour specimens there were seen an occasional large granular cast, erythrocytes and leukocytes. The

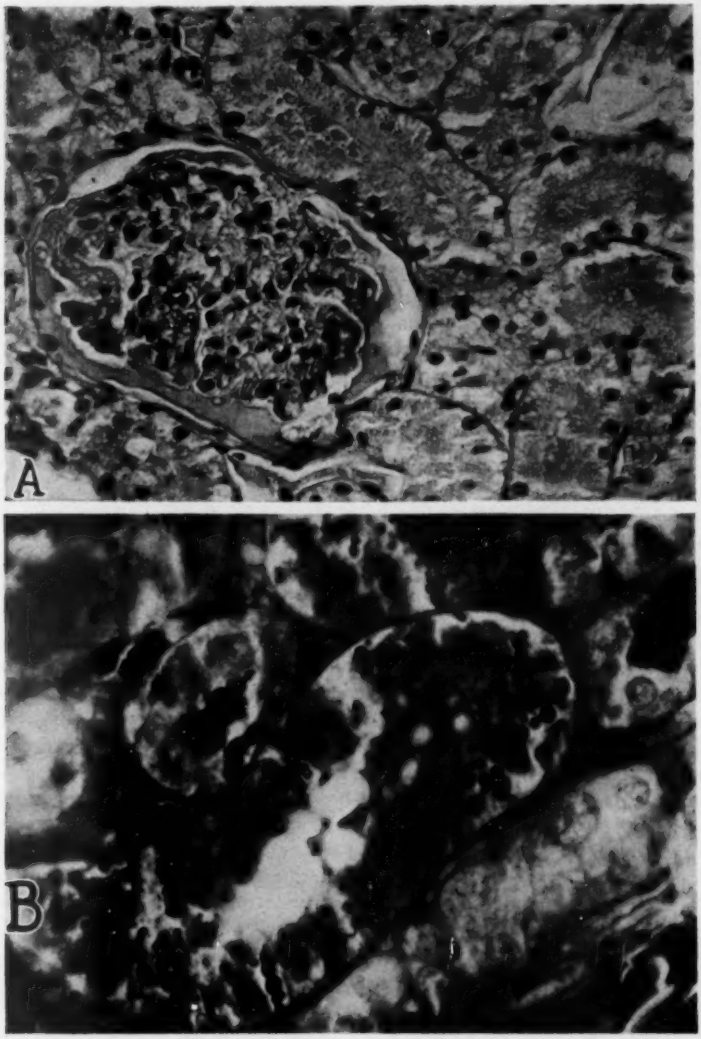

Fig. 10.—*A*, kidney removed five hours after injection of toxin, showing extensive hemolysis in Bowman's space and severe destruction of tubules (hematoxylin and eosin).

B, higher magnification of *A* (× 800). Note extensive and severe destruction of mitochondria and the presence of hemoglobin in the lumens of the tubules and in the cell.

subsequent specimens presented an increasing number of granular casts, erythrocytes, leukocytes, amorphous granules and debris.

The hematocrit readings in this group revealed an increase in the column of cells for three hours after injection; then, with the increasing hemolysis, the cell column declined.

The histologic examination of the kidney four hours after injection (fig. 9) revealed swelling of a great number of the glomerular tufts and hemoglobin in Bowman's space. Hemoglobin was also seen in some of the capillaries, while others showed congestion. Many of the proximal convoluted tubules presented a normal appearance, while others showed varying degrees of cloudy swelling and vacuolation. Some of the sections showed areas of tubular necrosis. In some of the tubules, small globules of hemoglobin were seen to have been absorbed within the cells. The most striking alterations were the severe and diffuse mitochondrial changes. In many cells the mitochondria had disappeared; in others they were clumped together.

Considerable amounts of ferrocyanide were seen in some of the glomeruli and proximal convoluted tubules. In the frankly swollen glomeruli little to no ferrocyanide was observed. The phenol red was absent in the proximal convoluted tubules. In one of the animals examined five hours after the injection, evidence of advanced hemolysis was seen (fig. 10). Hemoglobin was also seen in the proximal convoluted tubules and in portions of Henle's loop. The mitochondrial changes were found to be the most severe and extensive in this experiment.

COMMENT

The intramuscular injection of Cl. perfringens toxin was followed after a two hour interval by rapid development of edema in the involved leg, which averaged 2 per cent of the body weight at the end of a four hour period; this was accompanied by gradual but definite hemoconcentration. As the average blood volume of the dog (in percentage of body weight) has been calculated as 7.2 per cent,[16] the considerable local edema in the extremity that received the toxin may account for the larger part of the hemoconcentration and the drop of the blood chlorides. The extrarenal factors and the diminution of renal function may be associated with the increased blood urea nitrogen and the diminished excretion of urinary chlorides. Similar results have been presented in part by Aub and co-workers [17] as following injections of various fractions of perfringens toxin, and by other investigators [14] as following crushing or ligation of an extremity. The rapid decrease in the volume of urine excreted one hour after the injection of perfringens toxin may in part be a consequence of the edema developing in the leg that received the injection, for it was not observed in the experiments in which a plaster cast was applied after the injection; or, in part, it may be the result of circulatory or other changes in the kidney as described by Trueta and associates [18] or to a combination of both. The decrease in volume of urine could not be correlated with the blood pressure.

No demonstrable histological alterations were visible in the kidneys until two and one-half to three hours after the injection of the Cl. perfringens toxin, at which time many of the glomeruli showed a diminution of circulating blood and beginning

16. Dukes, H. H.: The Physiology of Domestic Animals, ed. 5, Ithaca, N. Y., Comstock Publishing Company, Inc., 1942, p. 61.

17. Pope, Zamecnik and Aub.[2a] Zamecnik, Nathanson and Aub.[2b] Kety and others.[5]

18. Trueta, J.; Barclay, A. E.; Daniel, P. M.; Franklin, K. J., and Prichard, M. M. L.: Studies of Renal Circulation, Springfield, Ill., Charles C Thomas, Publisher, 1947.

swelling of the glomerular tufts. However, at this time, the amount of glomerular filtration, as indicated by the amount of ferrocyanide present in Bowman's space and in the tubules, did not appear to be diminished. Subsequently, increasing numbers of glomeruli became swollen, filling Bowman's space. Widespread vasoconstriction of the cortical arterioles and capillaries was observed. A marked diminution of the ferrocyanide filtering through the numerous swollen glomeruli was evident, while in normal-appearing glomeruli the filtration appeared to be normal. These observations were correlated in time with the development of the maximal edema of the involved extremity and could represent a vasomotor compensation to maintain the blood pressure and adequate circulation [2b] in the face of a rapid withdrawal of a large amount of fluid from the circulating blood. Between four and five hours after the injection the increasing circulatory failure became quite pronounced and manifested itself in the kidney in the wide dilation of glomerular capillaries, in the reduction in size of the glomerular tufts and in the generalized vascular congestion.

The intracellular alterations manifested by the mitochondria were among the earliest observed. These at first involved scattered cells of the proximal convoluted tubules. In the later specimens, more severely affected areas were encountered. Even in areas that were extensively and actively involved there was persistence of cells that appeared normal or nearly normal. The intracytoplasmic alterations observed in some of the sections when special technics were used were more pronounced than those seen with the routine staining technics. The histochemical studies employing phenol red, which is excreted chiefly by the proximal convoluted tubules, revealed that the cells were not uniformly affected. In the early stages some of the tubules were more involved than others, and in the later stages practically all the tubules were affected and failed to secrete the dye within their cells.

In the proximal convoluted tubules a correlation was observed between the structural appearance of the mitochondria and the function of the tubules as indicated by the secretion of phenol red. The amount of dye secreted appeared to bear an inverse ratio to the severity of the mitochondrial alterations.

As has been pointed out, the action of the toxin, whether injected intramuscularly or intravenously, appears to be the result of a combination of factors. 1. There is a lecithinase [19] component which apparently acts on the phosphatides of cell surfaces, hemolyzing erythrocytes and affecting or destroying tissue cells. 2. The combination of a lecithinase and a hyaluronidase component (plus amounts of unknown toxins) results in an edema-forming power.[20] 3. There is the pos-

19. Glenny, A. T.; Barr, M.; Llewellyn-Jones, M.; Dalling, T., and Ross, H. E.: Multiple Toxins Produced by Organisms of Clostridium Welchii Group, J. Path. & Bact. **37**:53, 1933. Zamecnik, P. C.; Folch, J., and Brewster, L.: Protection of Animals Against Clostridium Welchii (Type A) Toxin by Injections of Certain Purified Lipids, Proc. Soc. Exper. Biol. & Med. **60**:33, 1945.

20. (*a*) McLean, D., and Hale, C. W.: Study on Diffusing Factors: The Hyaluronidase Activity of Testicular Extracts, Bacterial Culture Filtrates and Other Agents that Increase Tissue Permeability, J. Biochem. **35**:159, 1941. (*b*) Oakley, C. L.: The Toxins of Clostridium Welchii: Critical Review, Bull. Hyg. **18**:781, 1943. (*c*) MacFarlane, R. G., and MacLennon, J. D.: The Toxemia of Gas Gangrene, Lancet **2**:328, 1945.

sibility that all the effects obtained are not due to the direct action of the toxin alone but may be ascribed in part to the products of tissue breakdown.[21]

A sequence of glomerular and tubular alterations was observed following intramuscular injection of Cl. perfringens toxin which suggests that the action may be the result of (1) circulatory alterations, (2) the direct action of the toxin or toxic products on the cells or (3) a combination of these factors.

The "protective" effect of the plaster cast manifested itself in the delayed appearance of abnormal urinary constituents and in the normal volume of urine excreted. The tubular changes were slower in development and possibly a little less severe, although the disturbances of function manifested by the phenol red appear to be about the same. The application of the plaster cast, by limiting the local formation of edema and possibly by diminishing the diffusion of the toxin, diminished the vasospastic reaction of the glomerular and the cortical capillaries and delayed some of the changes and possibly may have diminished slightly some of the tubular alterations.

Injection of the toxin followed by massage was accompanied by increased hemolysis and by diffuse and severe intracellular derangement of the proximal convoluted tubules, observed in the disintegration of the mitochondria as well as in the absence of phenol red. Later there was severe and diffuse destruction of the tubular structure. The foregoing changes were reflected in the earlier appearance of abnormal urinary constituents. These striking renal changes appear to reflect the presence of an additional factor or combination of factors affecting the tubules, for the pattern of development differed in the other experiments. These alterations may be the result of the toxic action of the increased amounts of hemoglobin pigment absorbed by the tubules, or of the presence, in addition to the other toxic factors, of increased amounts of lecithinase and its action on the cell surfaces. In the late stages the glomerular tufts were occluded with hemoglobin. Bywaters [22] laid emphasis on the toxicity of myoglobin in the genesis of the anuria syndrome in that myoglobin was precipitated in the lumens of the distal convoluted tubules. The intracellular changes were not elucidated by Bywaters. Experimental hemoglobinemia and its effect on the kidney have been studied by a number of workers, and the effects are similar to those reported for myoglobin. The height of the hemoglobin level attained in the plasma affecting the tubular absorption appears to be the chief factor in determining the renal insufficiency, according to a recent report by Flink.[23]

SUMMARY

Lethal doses of Clostridium perfringens toxin injected intramuscularly produced extensive edema at the site of injection, hemoconcentration, alterations in the electrolytes of the blood, a gradual drop in blood pressure reaching the shock level and

21. MacLennon, J. D., and MacFarlane, R. G.: Toxin and Antitoxin Studies of Gas Gangrene in Man, Lancet **2**:301, 1945. MacFarlane and MacLennon.[20a] Zamecnik and others.[20b]

22. Bywaters, E. G. L.: Ischemic Muscle Necrosis: Crushing Injury, Traumatic Edema, the Crush Syndrome, Traumatic Anuria, Compression Syndrome; A Type of Injury Seen in Air Raid Casualties Following Burial Beneath Debris, J. A. M. A. **124**:1103 (April 15) 1944.

23. Flink, E. B.: Blood Transfusion Studies: III. The Relationship of Hemoglobinemia and of the p_H of the Urine to Renal Damage Produced by Injections of Hemoglobin Solutions into Dogs, J. Lab. & Clin. Med. **32**:223, 1947.

a significant reduction in the volume of urine secreted that could not be correlated with the blood pressure and alterations in the urinary constituents.

In the proximal convoluted tubules a correlation was observed between the structural appearance of the mitochondria and the secretion of phenol red, there being an inverse ratio between the amount of mitochondrial alterations and the amount of phenol red secreted.

A sequence of functional and structural alterations of both the glomeruli and the tubules was observed which suggested that the action of the toxin on the kidneys may be the result of (1) the circulatory alterations, (2) the direct action of the toxin or toxic products on the cells or (3) a combination of these factors.

The application of a plaster cast following intramuscular injection of Cl. perfringens toxin, by limiting the local formation of edema and possibly by diminishing the diffusion of the toxin, significantly reduced the hemoconcentration, maintained the urinary output, diminished the vasospastic phenomena of the glomerular and cortical capillaries and delayed the appearance of, and possibly modified some of, the tubular alterations of the kidney.

Increasing the rate of diffusion of the toxin by massage after intramuscular injection resulted in the following changes: increased hemolysis, earlier appearance of abnormal urinary constituents, alteration of the pattern of development of intracellular alterations in the tubules, as well as more diffuse and severe alterations, and more severe and extensive destruction of the kidneys.

ENDOPHLEBOHYPERTROPHY AND PHLEBOSCLEROSIS

I. The Popliteal Vein

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AND
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FOR THE past three years we have been engaged in a systemic study of the structural changes occurring in the large veins. In the initial phase of this work we became concerned with the problem of phlebosclerosis. The literature [1] on this subject is indicated in table 1. Review of these publications reveals that there is no agreement as to nomenclature, etiologic factors, pathogenesis and histopathological aspects of this process. Phlebosclerosis has been called venofibrosis, fibrous endophlebitis, hyperplastic phlebitis and hypertrophy of the veins.

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The Department of Pathology of Michael Reese Hospital is in part supported by the Michael Reese Research Foundation.

1. (*a*) Morgagni, J. B.: The Seats and Causes of Diseases Investigated by Anatomy, translated by B. Alexander, London, Millar and Cadell, 1769, Letter 44, art. 9, vol. 2, p. 601. (*b*) Baillie, M.: Transactions of a Society for the Improvement of Medical and Chirurgical Knowledge, **1**:119, 1793. (*c*) Lobstein, J. F.: Traité de l'anatomie pathologique, Paris **2**:555, 1833. (*d*) von Rokitansky, C. F.: Handb. d. path. Anat. **2**:651, 1844. (*e*) Thoma, R.: Virchows Arch. f. path. Anat. **204**:1, 1911. (*f*) Sack, E.: Ibid. **112**:403, 1888. (*g*) Fischer, B.: Beitr. z. path. Anat. u. z. allg. Path. **27**:494, 1900. (*h*) Kaya, R.: Virchows Arch. f. path. Anat. **189**:466, 1907. (*i*) Faber, A.: Die Arteriosklerose: Ihre pathologische Anatomie, ihre Pathogenese und Aetiologie, Jena, G. Fischer, 1912, p. 122. (*j*) Simmonds, M.: Virchows Arch. f. path. Anat. **207**:360, 1912. (*k*) Ljungdahl, M.: Untersuchungen über die Arteriosklerose des kleinen Kreislaufs, Wiesbaden, J. F. Bergmann, 1915. (*l*) Cramer, H.: Virchows Arch. f. path. Anat. **230**:46, 1921. (*m*) Benda, C.: Venen, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie: Vol. 2, Herz und Gefässe, Berlin, Springer-Verlag, 1924, p. 786. (*n*) Schilling, W.: Virchows Arch. f. path. Anat. **263**:658, 1926. (*o*) McIndoe, A. H.: Vascular Lesions of Portal Cirrhosis, Arch. Path. **5**:23 (Jan.) 1928. (*p*) Allen, E. V., and Page, I. H.: Deutsches Arch. f. klin. Med. **168**:193, 1930. (*q*) Hauswirth, L., and Eisenberg, A. A.: Disseminated Venofibrosis (Phlebosclerosis): Its Clinicopathologic Significance, Arch. Path. **11**:857 (June) 1931. (*r*) Moschcowitz, E.: Contribution to the Medical Sciences in Honor of Dr. Emanuel Libman, New York, International Press, 1932, vol. 2; Vascular Sclerosis, London, New York, Toronto, Oxford University Press, 1942. (*s*) Weeber, J.: Virchows Arch. f. path. Anat. **292**:75, 1934. (*t*) Gross, H.: Sclerosis of Hepatic Veins in Chronic Congestive Heart Failure, Arch. Path. **23**:457 (April) 1937. (*u*) Waaler, E.: Am. J. Path. **13**:855, 1937. (*v*) Gross, H., and Handler, B. J.: Sclerosis of Superior Vena Cava in Chronic Congestive Heart Failure, Arch. Path. **28**:22 (July) 1939. (*w*) Pei-Lin Li: J. Path. & Bact. **50**:121, 1940. (*x*) Short, R. H. D.: Ibid. **50**:419, 1940. (*y*) Tedeschi, C.: Anat. Rec. **79**:243, 1941. (*z*) Carere-Comes, O.: Cardiologia **4**:283, 1940; Folia cardiol. **2**:265, 1941; Cuore e circolaz. **25**:106, 1941.

TABLE 1.—*Historical Review of Phlebosclerosis*

Author	Year	Vein Studied	Contribution
Morgagni [1a]	1709	Inferior vena cava	Calcification of inferior vena cava
Baillie [1b]	1793	Inferior vena cava	Calcification of vein where it bifurcates into iliac veins
Lobstein [1c]	1833	General study	First used term "phlebosclerosis"; noted thickening of veins of lower extremities with age
von Rokitansky [1d]	1844	General study	Phlebosclerosis due to stasis and inflammation
Thoma [1e]	1911	General study	Angiosclerosis due to disturbance of nutrition of media, primary or secondary to disease of vasa vasorum; weakening of media leads to local dilation, with slowing of blood, followed by proliferation of connective, elastic and muscle tissue of intima, and thickening of media, then secondary fatty degeneration
Sack [1f]	1888	General study	Agreed with Thoma as to pathogenesis; histological process consists of (1) proliferation of subendothelial connective tissue, (2) increase in intercellular substance and (3) loss of muscle and elastic fibers; diffuse and localized forms
Fischer [1g]	1900	General study	Chronic inflammation cause of phlebosclerosis; to previous description, Fischer added growth of elastic fibers in intima with thickening of media
Kaya [1h]	1907	General study	Denied inflammation and back pressure were causes; cause, local stress; process intimal, not medial; hyperplastic proliferation of connective, muscle and elastic tissue of intima; later replacement of muscle cells; rarely calcification and fatty degeneration; media thickened early —later atrophied
Faber [1i]	1912	Inferior vena cava, portal vein	Inflammation, primary cause; stasis, secondary; observed calcification of elastic fibers in media
Simmonds [1j]	1912	Portal vein	Primary type due to syphilis; secondary, to stasis
Ljungdahl [1k]	1915	Pulmonary vein	Sclerosis in mitral stenosis; arteriosclerosis and phlebosclerosis same
Cramer [1h]	1921	Inferior vena cava	Redescribed plaques at bifurcation into iliac veins; local mechanical factor
Benda [1m]	1924	General study	Not a single entity but end stage of degenerative or inflammatory disease; recognized senile type
Schilling [1n]	1926	General study	Redescribed Schilling-Cramer plaques; local disease, but related to arteriosclerosis; agreed with Kaya—intimal disease; found fatty degeneration in inferior vena cava and femoral vein and calcification in inferior vena cava
McIndoe [1o]	1928	Portal and hepatic veins	Sclerosis of portal and hepatic veins in portal cirrhosis, due to stasis
Allen and Page [1p]	1930	Inferior vena cava	No change in heart failure
Hausworth and Eisenberg [1q]	1931	Veins of lower extremities	"Venofibrosis"—loss of endothelium; hyalinization of denuded surface; hyperplasia of connective tissue of media, less of intima; relationship to phlebosclerosis not clear
Moschcowitz [1r]	1932 1942	Hepatic and pulmonary veins	Back pressure primary cause in heart failure; lesions identical with arteriosclerosis
Weeber [1s]	1934	Portal and omental veins	Sclerosis in cirrhosis and heart failure
Gross [1t]	1937	Hepatic vein, inferior vena cava	Thickening of walls with deposition of plaques and longitudinal streaks; agreed with Kaya's description of histological appearance; due to increased venous pressure
Waaler [1u]	1937	Superior vena cava	Rheumatic and nonrheumatic changes in rheumatic fever
Gross and Handler [1v]	1939	Superior vena cava	Sclerotic changes in congestive heart failure
Li [1w]	1940	Portal, splenic and mesenteric veins; inferior vena cava	In cardiac failure, emphysema and cirrhosis—thickening of intima, development of longitudinal muscle fibers in subendothelial region, muscular hypertrophy of media
Short [1x]	1940	General study	Vasa vasorum in intima in phlebosclerosis
Tedeschi [1y]	1941	General study	Fibromuscular thickening of walls of veins adjacent to arteries; considered normal
Carere-Comes [1z]	1941	General study	Distinguished between active venous hypertension in mitral and tricuspid stenosis, and passive venous hypertension in heart failure; hypertrophy of circular muscle of media in former, not in latter; more plaques in former

It has been considered to be due to inflammation, syphilis, degenerative diseases, venous stasis, active venous hypertension or local mechanical stress. It has been thought by some to be a generalized disease and by others a localized disease. Some authors have considered it a process similar to arteriosclerosis, while others have separated the two conditions. The primary pathological change has been placed in the intima by some and in the media by others. Very few workers have attempted to differentiate the changes in phlebosclerosis from the normal histological variations of the veins or from changes that are associated with advancing age.

GENERAL STATEMENT OF THE PROBLEM

In this study we have been cognizant of the different gross and histological patterns in the various veins as well as of the different mechanical and hydrodynamic stresses to which they are subjected. Thus it was necessary to study each vein separately and then restudy the entire subject of "phlebosclerosis" from the standpoint of the common denominators seen, as well as from that of the comparative differences of various veins.

TABLE 2.—*Popliteal Veins Studied According to Age Groups*

Age	Number
0-10	17
11-20	3
21-30	4
31-40	10
41-50	10
51-60	21
61-70	41
71-80	16
81-90	5
Total	127

In the study of each vein the same course was followed: A study was made of (1) the functional gross anatomy of the vein, (2) the gross structure of the vein and its changes with advancing age (excluding cases with back pressure), (3) the microscopic structure of the vein and its changes with age (excluding cases with back pressure) and (4) a comparison of the gross and microscopic structure of the veins, with and without back pressure.

The following veins were or are being studied: (1) popliteal vein, (2) external and common iliac veins, (3) inferior vena cava, (4) pulmonary, (5) hepatic, (6) portal, (7) splenic, (8) mesenteric and (9) innominate veins, (10) superior vena cava and (11) renal, (12) femoral, (13) saphenous and (14) posterior tibial veins. The present report deals with the popliteal vein.

MATERIAL AND METHODS

The popliteal veins of persons routinely coming to autopsy were dissected in their entirety from their origin in the opening in the adductor magnus muscle to the entry of the anterior tibial veins. One hundred and twenty-seven veins were thus obtained from patients of various age (table 2). The veins were opened immediately after removal, and their gross structure was carefully noted. They were then fixed in solution of formaldehyde U. S. P. diluted 1:10, and numerous sections were taken in the longitudinal and transverse planes. These were embedded in paraffin and then stained routinely with hematoxylin and eosin and with Weigert's elastica stain counterstained with Van Gieson's connective tissue stain. Some sections were stained with

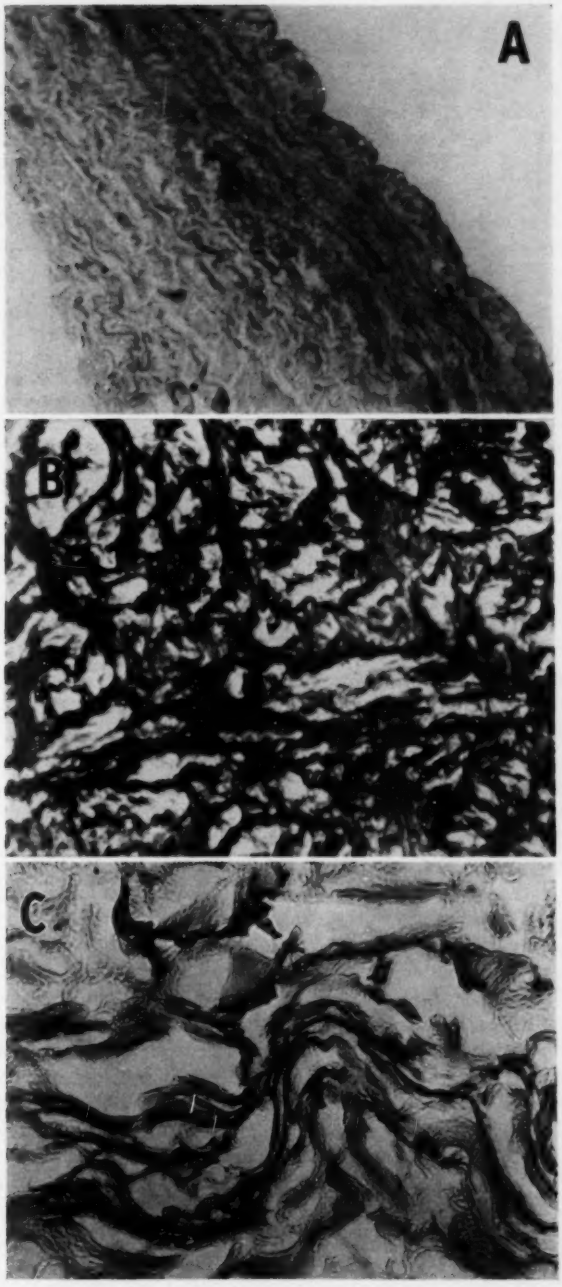

Fig. 1.—*A*, photomicrograph of cross section of popliteal vein. McManus-Lillie-Hotchkiss glycoprotein stain; × 100.

B, photomicrograph of basement membranes around muscle fibers. Gomori's reticulum stain (untoned); × 400.

C, photomicrograph of basement membranes around elastic fibers. Gomori's reticulum stain (untoned); × 240.

orcein, Heidenhain's azocarmine, Masson's trichrome, the McManus-Lillie-Hotchkiss glycoprotein stain for ground substance and basement membranes, Gomori's stain for reticulum (with and without toning), Lillie's modification of Langeron's alizarin red S stain for calcium, and Sudan IV for fat. In addition, portions of four veins were prepared by freezing and drying by the Altmann-Gersh technic and embedded in paraffin. Sections were then stained with hematoxylin and eosin, and for glycoproteins. Other sections were kept in 95 per cent alcohol overnight before staining for glycoprotein.

The procedure employed in the study of ground substance and basement membrane was based on the work of Gersh and Catchpole.[2] These investigators, studying ground substance and basement membranes in various tissues, utilized the procedure devised independently by McManus [3] and Lillie [4] and given chemical significance by Hotchkiss,[5] for the staining of water-insoluble and alcohol-insoluble glycoproteins. Gersh and Catchpole believe ground substance to consist basically of glycoproteins organized on a submicroscopic or molecular level. Basement membrane represents either a condensation of ground substance or an aggregate of glycoproteins in a state of polymerization different from that of ground substance. Basement membranes are interposed between the connective tissue and the ectodermal, endodermal and mesodermal derivatives. Within the glycoproteins are embedded reticular fibers of the connective tissue.

In the large veins, in formaldehyde-fixed tissue with glycoprotein stain, the ground substance is discernible only as a pale purple background. The basement membranes, however, are sharp, vivid purple lines. Such membranes stain deeply around muscle fibers and less prominently around elastic fibers and beneath the endothelium (fig. 1 *A*). With Gomori's reticulum stain the basement membrane about the muscle presents a heavy reticular network (fig. 1 *B*), while that around the elastic fibers consists of sharp linear formations with bridging nets (fig. 1 *C*).

FUNCTIONAL GROSS ANATOMY OF THE POPLITEAL VEIN

The popliteal vein is formed by the confluence of the anterior and posterior tibial veins at the distal border of the popliteus muscle (fig. 2). Two veins are thus formed (less frequently one) which unite to form one vein in the region of the origin of the two heads of the gastrocnemius muscle. The single popliteal vein thus formed lies in the popliteal fossa and extends to the opening in the adductor magnus muscle, where it assumes the name femoral. The dual popliteal veins lie to each side and slightly posterior to the popliteal artery. The single popliteal vein likewise lies posterior to the popliteal artery but shifts its position as follows: In the lower part of the fossa it is slightly medial to the artery. It then crosses obliquely to lie lateral to the artery in the proximal part of the fossa. The popliteal veins are tightly bound to the popliteal artery in a fascial sheath. The popliteal vein is covered posteriorly by the tibial nerve, which lies lateral to the vein above, and medial to the vein below. The vein is covered proximately by the semimembranosus muscle, in the center of the popliteal space by the skin, superficial and deep fascia, and distally by the two heads of the gastrocnemius muscle.

In our series the valvular arrangement of the popliteal vein was found to be as follows: A bicuspid valve guarded the junction of the popliteal and femoral veins in all instances. In 16 per cent of the cases one and rarely two bicuspid or atretic valves were present in the supracondylar portion. In the remainder no valves were found from the junction of the two popliteal veins to the beginning of the femoral.

2. Gersh, I., and Catchpole, H. R.: Am. J. Anat. **85**:457, 1949.
3. McManus, J. F. A.: Nature, London **158**:202, 1946; Stain Technol. **23**:99, 1948.
4. Lillie, R. D.: Histopathologic Technic, Philadelphia, The Blakiston Company, 1948.
5. Hotchkiss, R. D.: Arch. Biochem. **16**:131, 1948.

Where the popliteal vein formed two collaterals in its lower portion, each collateral usually presented from one to three bicuspid valves. Occasionally only one collateral contained valves. Where the popliteal vein was a single vessel in its lower portion, it usually contained one and sometimes two bicuspid valves. In general, the valvular arrangement of the popliteal veins was markedly variable.

From the functional point of view, the popliteal vein is subject to considerable hydrostatic pressure on standing. This is superimposed on the relatively high pressure related to its distance from the heart. Hence the vein possesses a thick wall, almost equal to that of the artery. In addition it is subject to the local stresses created by the bend of the knee.

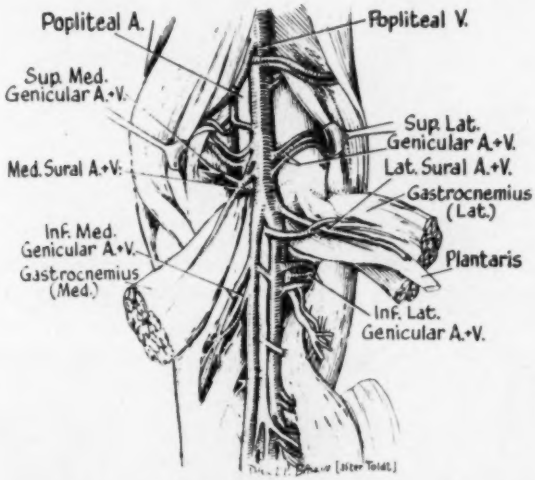

Fig. 2.—Diagram of the gross anatomy of the popliteal vein (after Morris, H.: Morris' Human Anatomy; Complete Systematic Treatise, edited by C. M. Jackson, ed. 8, Philadelphia, Blakiston's Son & Company, 1925).

GROSS STRUCTURE AND ITS CHANGES WITH AGE

The vein after being removed from the body contracts in both longitudinal and transverse planes. Thus the gross appearance of the vein during life is modified by the factor of contraction. The normal color of the vein varies from pinkish gray to yellowish pinkish-gray to grayish pink. On close inspection numerous fine, more or less parallel longitudinal striations are noted, which in turn are arranged in bands. These slightly elevated longitudinal bands are parallel in regions between tributaries but are divergent or convergent at the sites of tributaries. The fine and coarse striations are produced by the postmortem contraction of the circular muscle of the media, thus corrugating the longitudinal elements of the three layers, especially the muscle fibers. The convergence or the divergence of the larger bands is related to the incorporation of longitudinal muscle fibers of tributaries into those of the main vein.

The above described basic structure shows striking changes with increasing age. With increasing age the wall becomes thicker, and the coarse and fine linear

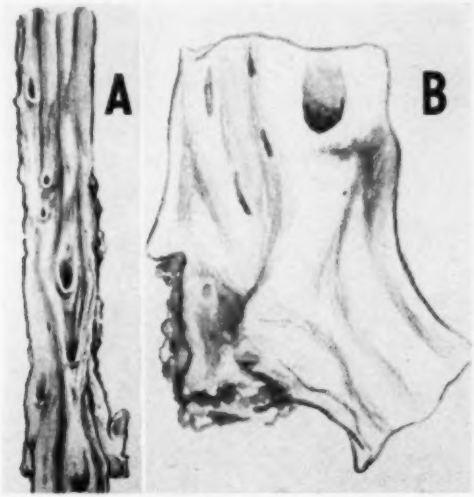

Fig. 3.—Diagrammatic accentuated drawings of thickenings related to entries of tributaries: *A*, early stage. *B*, advanced stage.

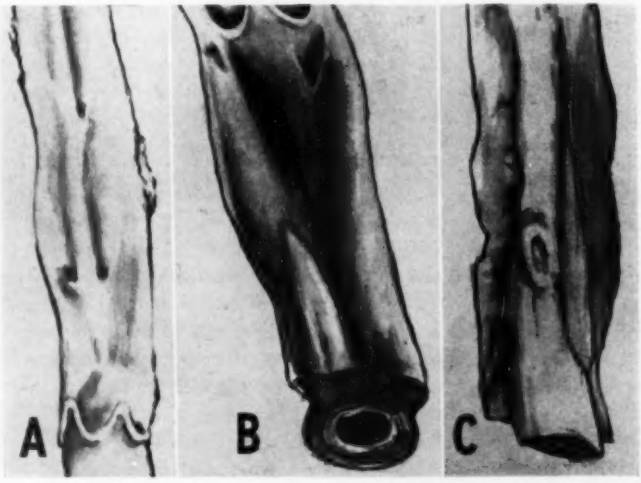

Fig. 4.—Diagrammatic accentuated drawings of thickenings situated in the juxta-arterial position: *A*, early stage. *B*, advanced stage. *C*, calcific crater in advanced plaque.

striations become accentuated. Shortly after birth, linear streaks or thickenings appear at the mouths of tributaries (fig. 3 *A*). At the age of 2 a longitudinal linear thickening can be seen in that part of the vein just to one side of its point of

maximal contact with the artery (fig. 4 *A*). Up to about the age of 15 or 20 these thickenings are smooth and only slightly whitened. Progressively thereafter they become broader and higher. They take on irregular furrows in oblique directions, producing a more geographic, fenestrated or cobblestone appearance (figs. 3 *B* and 4 *B*). Their color becomes yellow, white or yellow-white, or they are punctuated by yellow dots and streaks. They take on increased firmness, so that specimens of subjects 50 to 60 years of age may feel cartilaginous. In a few instances, actual calcific craters up to 3 mm. in greatest dimension may lie in these thickenings (fig. 4 *C*).

In the younger age groups all these thickenings are related directly either to the entry of tributaries or to the proximity of the popliteal artery. With increasing age they fuse, producing a more diffuse mosaic of irregular white thickenings. Their relationship, however, to tributaries and to the artery can still be discerned.

The lesions are seen mostly in the condylar and supracondylar portions of the vein, although they may extend throughout its entire length. In a few instances they are more obvious in the infracondylar portion. In the condylar region an irregular white cobblestone appearance is common, owing to the presence of numerous tributaries, and possibly to the bend of the knee. There is no obvious relationship between the number of thickenings and streaks, and the presence or the absence of valves.

MICROSCOPIC STRUCTURE AND ITS CHANGES WITH ADVANCING AGE

At birth the popliteal vein consists of an intima, a media and an adventitia (fig. 5). The intima consists of an endothelial lining beneath which there is an internal elastic lamella, the fibers of which are arranged only in a longitudinal direction. The media consists of transverse muscle cells, with elastic fibers showing a strong longitudinal component. The adventitia consists of elastic fibers which are mostly longitudinally oriented, with collagenous connective tissue fibers running in various directions, and occasional longitudinal muscle fibers. (According to Benninghoff,[6] the collagenous and elastic fibers are arranged in spirals.) A basement membrane consisting of a glycoprotein component with no reticulum accompanies the internal elastic lamella. The muscle cells have basement membranes consisting of fine but sharp glycoprotein linear formations and a heavy network of reticulum. The basement membranes of the elastic fibers likewise consist of sharp glycoprotein lines but have a less pronounced reticular network. All the elements mentioned are of course embedded in ground substance.

At birth, corresponding to the plaques seen grossly, on the luminal side of the internal elastic lamella there is found focally a proliferation in a longitudinal direction of a varying amount of elastic, muscle and collagen fibers (fig. 6). Any one of these elements may predominate. The reticulum and glycoprotein components of the basement membranes accompany these structures. This process we term *endophlebohypertrophy*. This process will be described more fully later, but henceforth we shall use this term for convenience of description.

Up to 2 to 3 years of age there is a progressive increase in the extent of endophlebohypertrophy, so that in the spot of greatest involvement the thickness of the intima equals that of the media. In the meantime there are an increase in the

6. Benninghoff, A.: Blutgefässe und Herz, in Möllendorff, W. V.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Springer-Verlag. 1930, vol. 6, pt. 1.

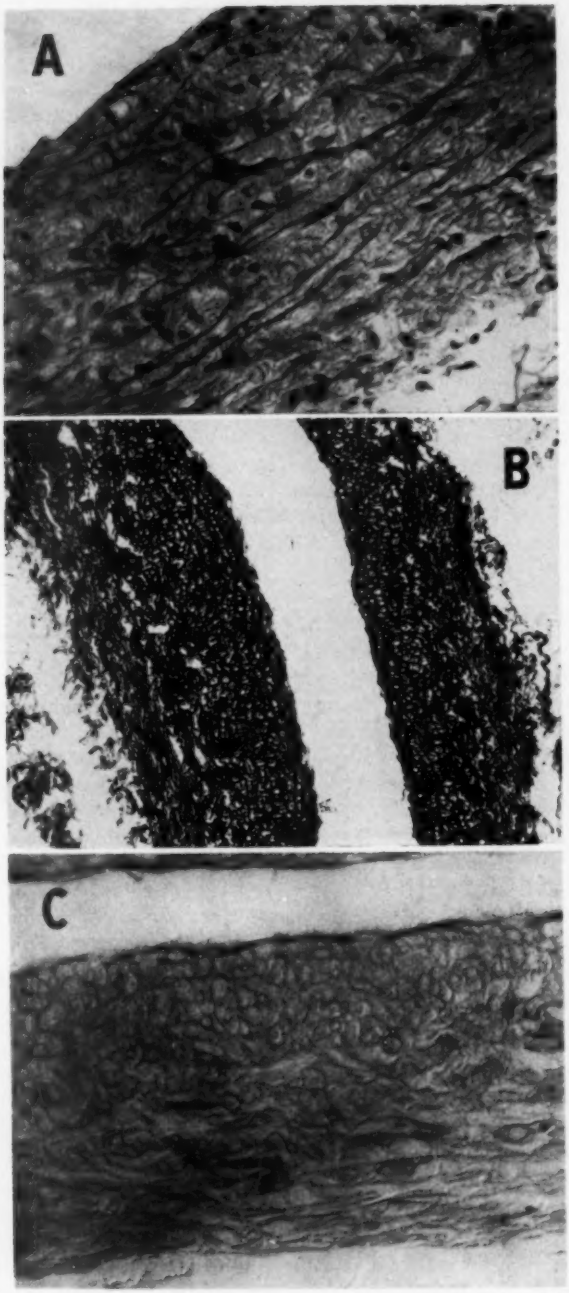

Fig. 5.—Photomicrographs of longitudinal sections of the popliteal vein at birth: *A*, Weigert's elastica-Van Gieson's connective tissue stain; × 200. *B*, Gomori's reticulum stain; × 120. *C*, McManus-Lillie-Hotchkiss glycoprotein stain; × 200.

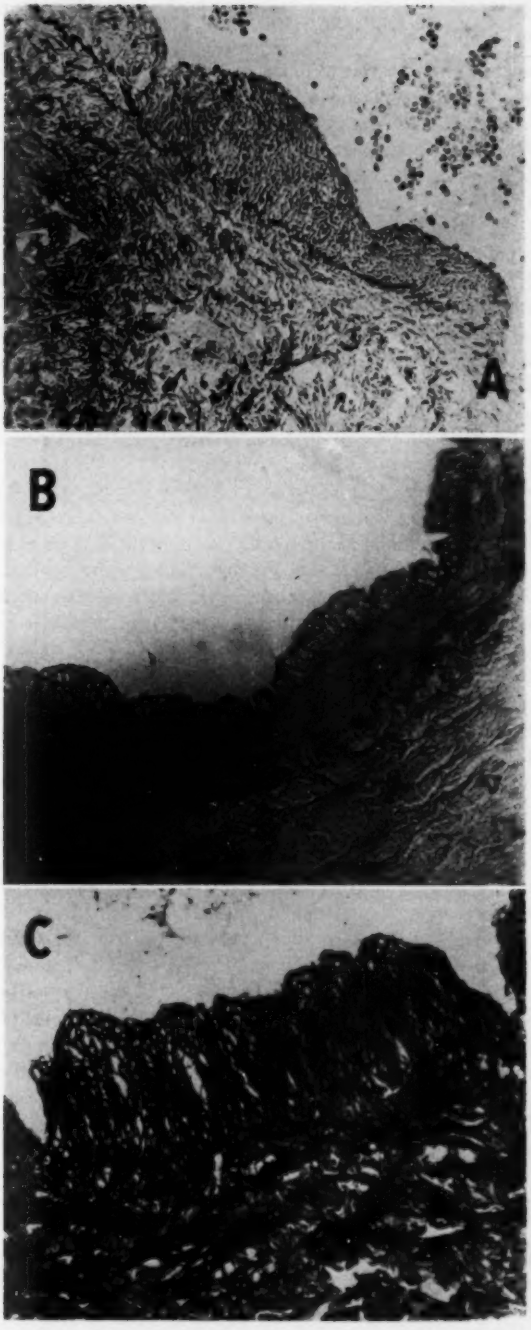

Fig. 6.—Photomicrographs of cross sections of the popliteal vein at birth showing areas of endophlebohypertrophy: *A*, Weigert's elastica-Van Gieson's connective tissue stain; × 120. *B*, McManus-Lillie-Hotchkiss glycoprotein stain; × 100. C, Gomori's reticulum stain; × 200.

amount and thickness of the elastic fibers of the entire wall, a distinct increase in the musculature of the media and a slight increase in the longitudinal muscle of the adventitia. At about 1 year of age the basement membrane beneath the endothelium acquires a reticular component, while the reticular component of the basement membranes of all the elastic tissue becomes sharper. Finally, the internal elastic lamella takes on a transverse component.

At 2 to 3 years of age there is apparently a diminution in the amount of endophlebohypertrophy. We do not know the reason for this. It may be related to the assumption of an upright posture. The vein at this age may therefore serve as a baseline for the study of the further development of changes (fig. 7 *A*). At this stage the endophlebohypertrophy is more or less limited to the regions about the entering tributaries and to the juxta-arterial position. It is now apparent that this process can involve the medial side of the internal elastic lamella as well as the luminal. When it does, the connective tissue component is considerable.

By the age of 10, and more clearly seen from 10 to 20, endophlebohypertrophy has again increased so that in spots it equals the media in thickness. It now takes on an added characteristic—a layering effect (fig. 7 *B*); an outer layer of hypertrophy is superimposed on an inner, separated by the internal elastic lamella. This lamella may or may not be noticeably reduplicated. The muscle cells and elastic fibers closer to the lumen take on a more oblique rather than the orderly longitudinal direction which is typical of the deeper layer. This process of alteration of pattern, with obliquity and irregularity of direction of muscle, connective tissue and elastic tissue components, we term *disorganization*. The media in regions of endophlebohypertrophy is increased in thickness.

In addition, at this time a distinct alteration of the ground substance occurs in the regions of endophlebohypertrophy (fig. 8 *A* and *B*). With hematoxylin and eosin, a light blue, somewhat vacuolated material is noted among muscle and elastic fibers in these regions. The material becomes a faint blue with Masson's trichrome stain, in contrast to the deeper blue of the collagen fibers. With the glycoprotein stain, more stainable glycoprotein is noted, with no increase in intensity of staining. This probably represents an increased amount of ground substance. Likewise the musculature of media and adventitia throughout the wall has increased in thickness over that present in the first decade.

In the third decade the process of endophlebohypertrophy is greatly accentuated, the intima exceeding the media in some regions. The process of disorganization described above now goes deeper into the area of endophlebohypertrophy. The process of layering is more prominent, with a greater amount of proliferation on the medial side of the internal elastic lamella. In these spots disruption of elastic fibers now becomes apparent (fig. 8 *C*). The alteration of the ground substance is also more prominent. With hematoxylin and eosin, one notes a greater vacuolation of the ground substance, with Masson's stain a decrease in blue stainability and with the glycoprotein stain a focal loss of stainable material (fig. 9). In addition, the basement membranes around muscle fibers may show either an increase or a decrease of stainability in their glycoprotein and reticular components (fig. 10).

In the fourth decade all the processes mentioned above are accentuated in the spots of endophlebohypertrophy. In addition, there is now a relative dearth of muscle fibers in these plaques as compared with other elements (fig. 11).

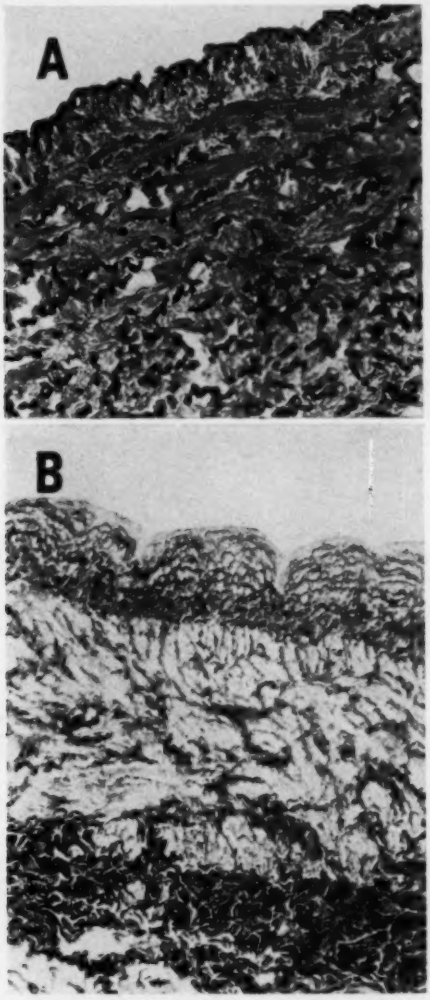

Fig. 7.—*A*, photomicrograph of a cross section of the popliteal vein at 2½ years of age. Note the medial type of endophlebohypertrophy. Weigert's elastica-Van Gieson's connective tissue stain; × 240.

B, photomicrograph of a cross section of the popliteal vein at 10 years of age. Note the layering effect in endophlebohypertrophy. Weigert's elastica-Van Gieson's connective tissue stain: × 120.

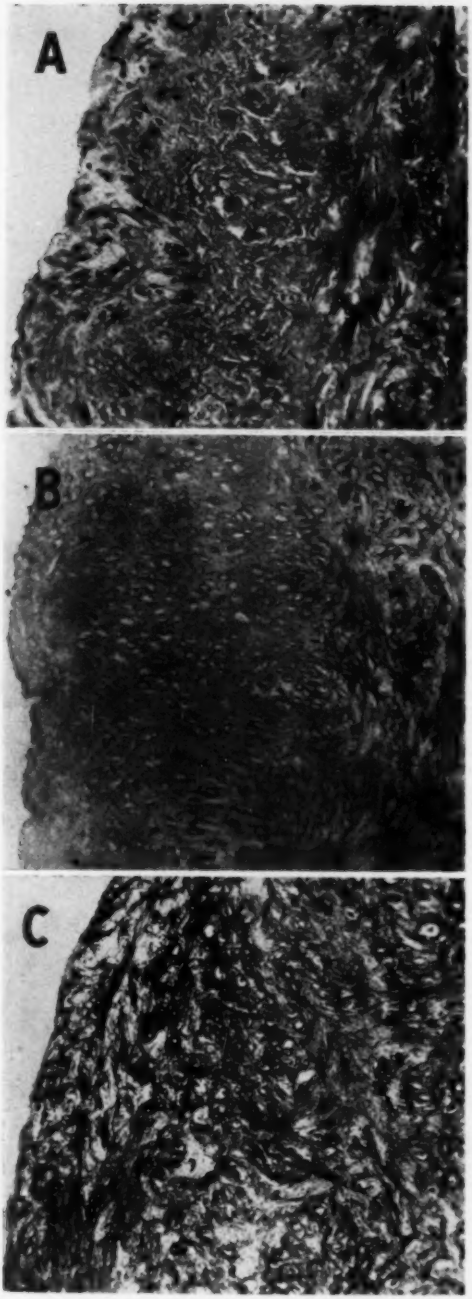

Fig. 8.—*A* and *B*, photomicrographs of cross sections of the popliteal vein at 10 years of age, showing early changes in the ground substance in areas of endophlebohypertrophy: *A*, hematoxylin and eosin stain; × 240. *B*, McManus-Lillie-Hotchkiss glycoprotein stain; × 100.

C, photomicrograph of a cross section of the popliteal vein, showing disruption of elastic fibers, in areas of endophlebohypertrophy. Weigert's elastica-Van Gieson's connective tissue stain; × 240.

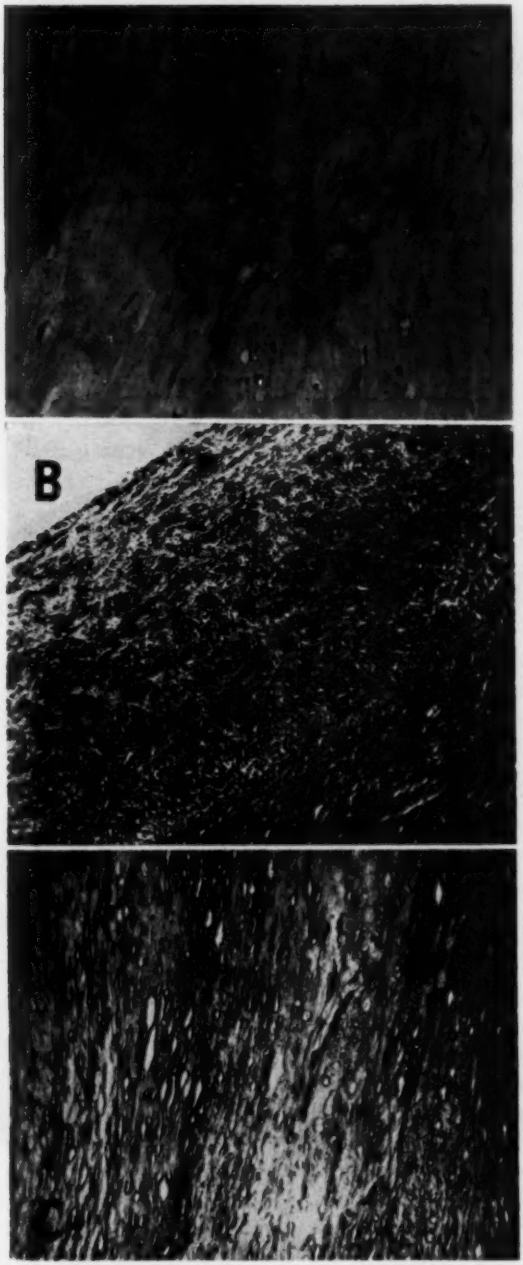

Fig. 9.—Photomicrographs of the popliteal vein showing advanced changes in the ground substance in areas of endophlebohypertrophy: *A*, longitudinal section; hematoxylin and eosin stain; × 200. *B*, cross section; Masson's trichrome stain; × 100. *C*, longitudinal section; McManus-Lillie-Hotchkiss glycoprotein stain; × 100.

This is accompanied with a relative increase of connective tissue in the intima. In some spots of endophlebohypertrophy the intima reaches two to three times the thickness of the media. The media beneath a spot of endophlebohypertrophy is now

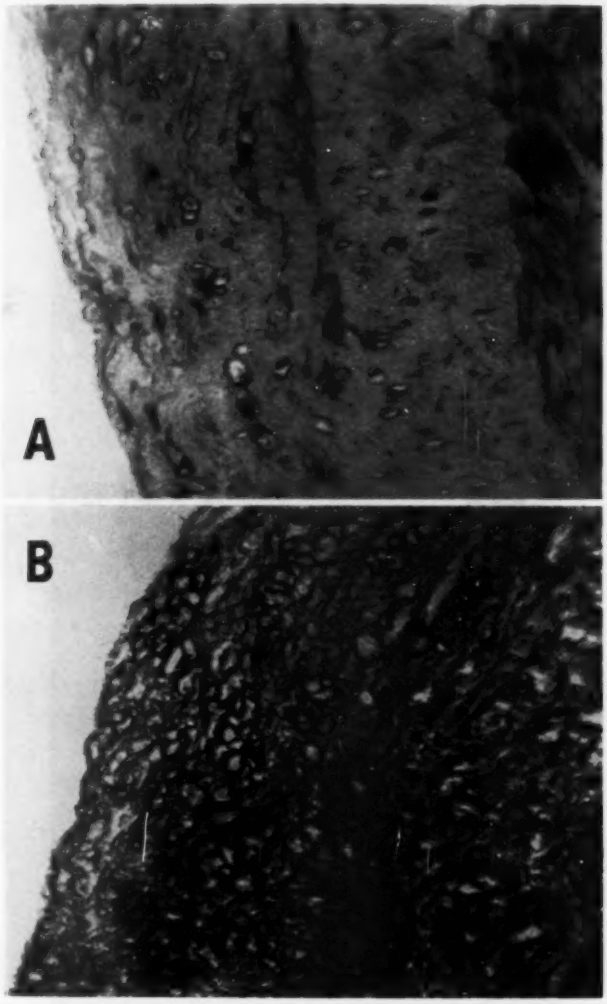

Fig. 10.—Photomicrographs of cross sections of the popliteal vein showing changes in the glycoprotein and reticular components of the basement membranes around muscle fibers in areas of endophlebohypertrophy: *A*, McManus-Lillie-Hotchkiss glycoprotein stain; × 200. *B*, Gomori's reticulum stain; × 200.

distinctly decreased in thickness because of a loss of all elements, especially the muscle cells.

In the fifth decade there are greater disorganization and excavation of the media. In the sixth decade, in addition to further progression in the previous findings, the plaques of endophlebohypertrophy show a distinct increase of connective tissue (fig. 12 *A*). Empty lacunas are now in evidence which are seen to be confluent areas of vacuolation of the ground substance (fig. 12 *B*). Adjacent to such areas the ground substance may show an increase of stainability in glycoprotein as well as a decrease (fig. 9 *C*). There is a marked loss of elastic fibers, and occasional spots of hyalinization are found in the connective tissue. Now for the first time there is noted a fine dusting of calcium granules in regions of endophlebohypertrophy. An occasional vein presents a localized, relatively large area of calcium deposit in the intima (fig. 13 *A* and *B*). These areas are in zones of

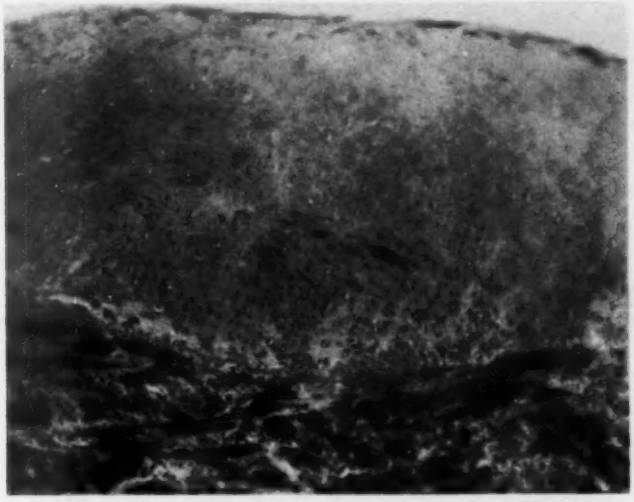

Fig. 11.—Photomicrograph of a cross section of the popliteal vein, showing loss of muscle cells and excavation of the media in areas of endophlebohypertrophy. Heidenhain's azocarmine stain; × 200.

increased connective tissue, and elastic fibers are abruptly interrupted at their margins.

The most impressive changes are found in the seventh decade. The process of endophlebohypertrophy is now more generalized. Muscle and elastic fibers are scant in the sea of connective tissue in the plaques of endophlebohypertrophy. Many of the muscle fibers assume a transverse direction. The florid lesions of endophlebohypertrophy (fig. 13 *C*) are from four to five times the thickness of the media, and the excavation of the media in these regions is almost complete. There may also be a focal increase of connective tissue in the media in regions of endophlebohypertrophy. In addition to changes in the basement membranes previously described, one occasionally notes irregular thickening and dissolution of the reticular and glycoprotein components of the basement membrane around the internal elastic lamella (fig. 14 *B*). The latter may be interrupted or definitely splintered (fig. 14 *A*).

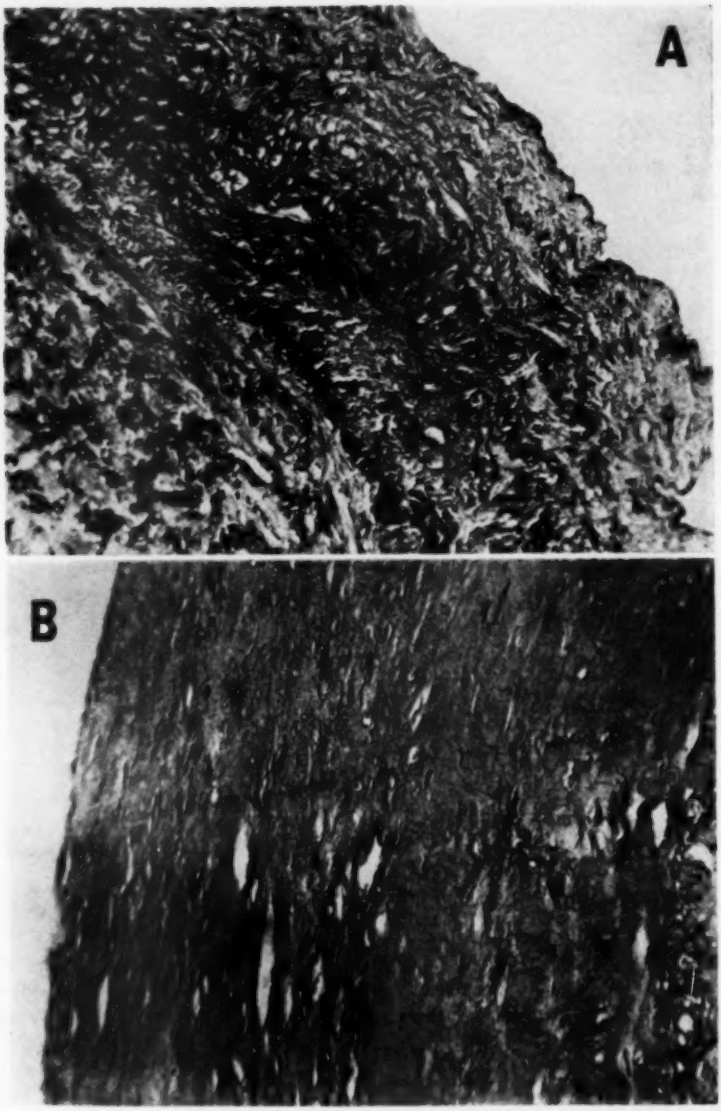

Fig. 12.—*A*, photomicrograph of a cross section of the popliteal vein showing an increase of connective tissue in areas of endophlebohypertrophy. Weigert's elastica-Van Gieson's connective tissue stain; × 200.

B, photomicrograph of a longitudinal section of the popliteal vein showing lacunar formation in areas of endophlebohypertrophy. Hematoxylin and eosin stain; × 100.

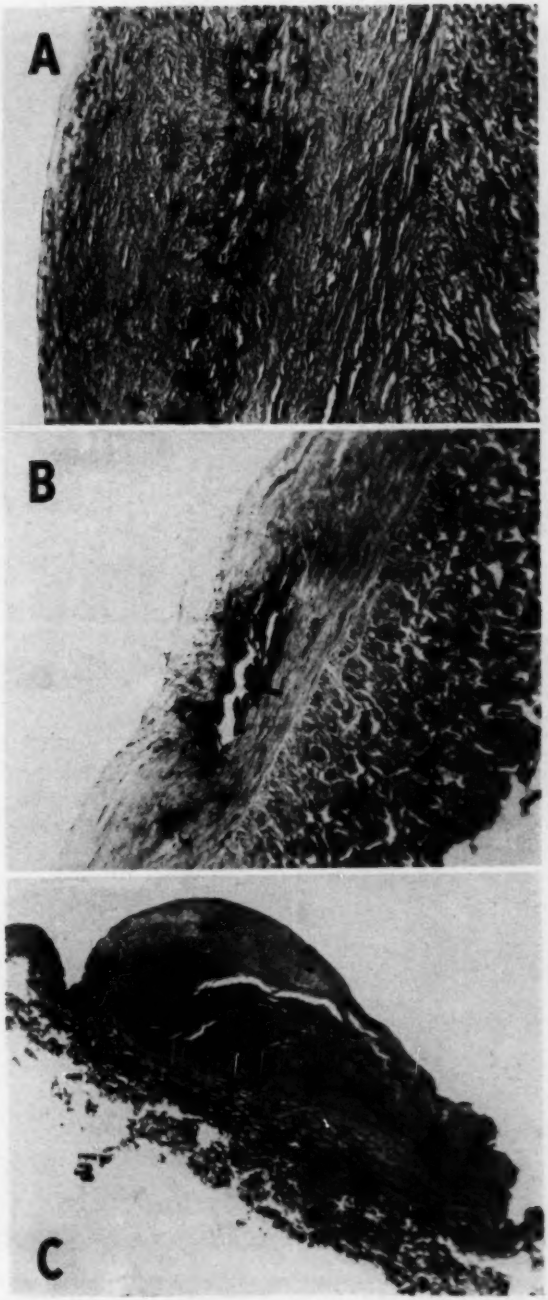

Fig. 13.—*A* and *B*, photomicrographs of cross sections of the popliteal vein showing calcium deposits in areas of endophlebohypertrophy: *A*, fine dispersal; hematoxylin and eosin stain; × 120. *B*, localized deposits; hematoxylin and eosin stain; × 120.

C, photomicrograph of a cross section of the popliteal vein showing a florid lesion of endophlebohypertrophy. Weigert's elastica-Van Gieson's connective tissue stain; × 21.

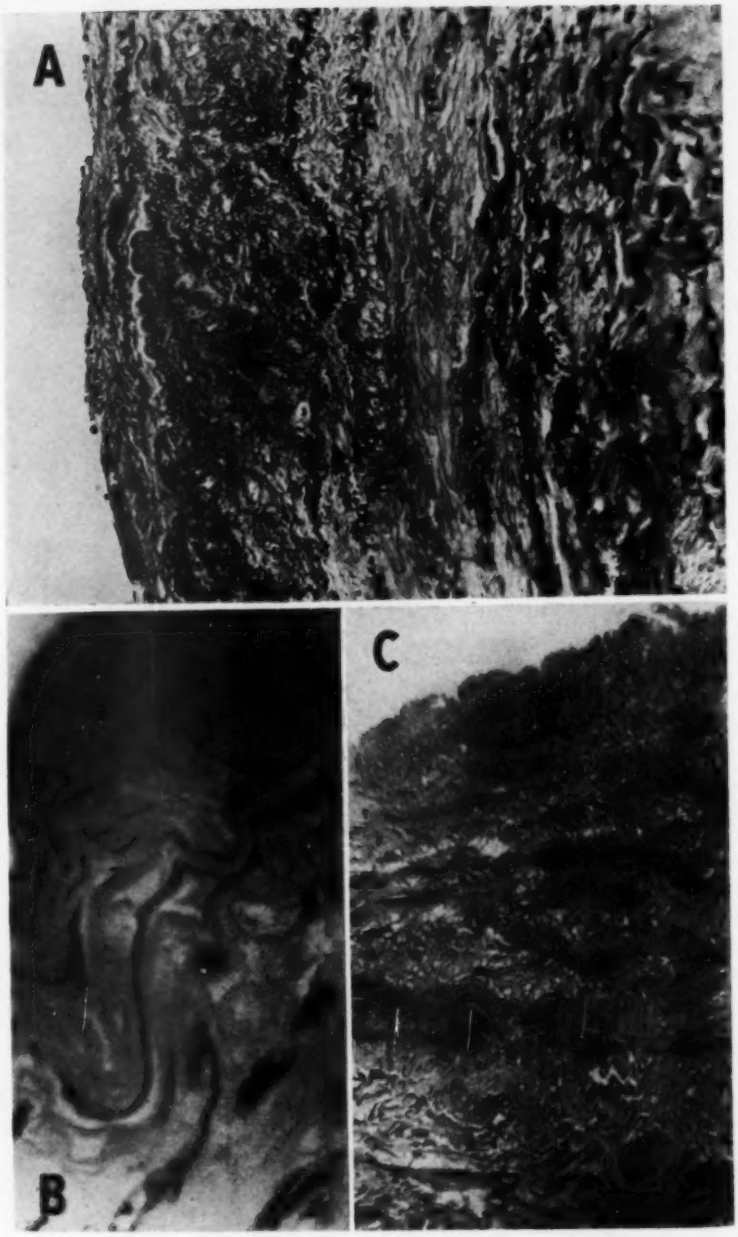

Fig. 14.—Photomicrographs of cross sections of the popliteal vein: *A*, dissolution of the internal elastic lamella; elastica stain; × 100. *B*, dissolution of the glycoprotein component of the basement membrane around the internal elastic membrane; glycoprotein stain; × 400. *C*, decrease of muscle fibers and an increase of collagenous connective tissue in old age; hematoxylin and eosin stain; × 100.

In the eighth and ninth decades there is no further increase of endophlebohypertrophy or its accompanying degenerative changes. In the media, however, there is a greater diffuse increase of connective tissue (fig. 14 *C*).

No fat was found in any of the lesions of endophlebohypertrophy at any age. Even in areas of calcification there was no trace of fat.

Comparison of veins with and without systemic evidence of right ventricular failure respecting the above gross and histological changes revealed no appreciable difference between the two groups. However, the first three decades of life were represented by too few cases with back pressure for one to reach a conclusion for the younger age groups.

CORRELATION OF GROSS AND MICROSCOPIC CHANGES

Histological examination of the longitudinal thickenings seen grossly adjacent to the popliteal artery and at the entries of tributaries reveal them to be areas of endophlebohypertrophy. There is no difference in basic histological structure between the lesions seen at the two sites. Those thickenings observed in the younger age groups (figs. 3 *A* and 4 *A*) which are smooth and pale, white or yellow-white show no further changes. The more irregular, whiter, more geographic thickenings, seen progressively with age (figs. 3 *B* and 4 *B* and *C*), histologically reveal disorganization, disruption of the elastic fibers, loss of muscle cells and an increase of connective tissue, in addition to changes in the ground substance and the basement membranes. Areas of endophlebohypertrophy seen microscopically are not always discernible grossly, but if they are of any size and depth, they are seen. After the age of 2 to 3 the process of endophlebohypertrophy is not limited to entries of tributaries and the juxta-arterial region but is widely distributed in the intima. It is, however, maximal at those points. In addition to these changes, with advancing age there are more generalized changes, seen grossly as a progressive whitening of the veins and histologically as a diffuse relative increase in connective tissue and loss of muscle fibers.

INTERPRETATION OF CHANGES

We have up to this point described the changes observed in the gross and microscopic structure of the popliteal vein in its progression with age. Our task now is to determine which changes may be considered part of the "normal" process of aging of the vein and which are to be attributed to pathological reactions. It is apparent that the discussion of the pathological changes occurring in circulatory tissue must be couched in terms of changing reactivity, since this tissue is constantly reacting to hydrodynamic stimulation and hence is in a constant state of flux. It is for this reason that our discussion of the popliteal vein did not commence with a statement of the "normal" gross and histological structure of the vein. For this "normal" varies with age, and certain changes which may conventionally be considered pathological may be "normal" aging phenomena.

We therefore make the following assumptions: 1. All retrogressive changes are pathological regardless of age. 2. A proliferative change is normal if it is the usual reaction of the tissue at a given age to a usually present stimulus. A corollary to this second point is: 3. A proliferative change is abnormal when it is an excessive reaction of the tissue at a given age to a usual stimulus, or a

usual reaction of the tissue to an unusual stimulus. Furthermore, we shall define phlebosclerosis as a process of noninflammatory retrogressive change occurring in a vein.

With these assumptions, the nature of the changes may now be interpreted. We pointed out that certain constant linear thickenings are seen grossly at the points of entry of tributaries and adjacent to the popliteal artery. It is clear that these are points of hydrodynamic stress. The reaction induced by this stress is endophlebohypertrophy. Histologically, endophlebohypertrophy consists in a proliferation, in the direction of blood flow, of the elastic, muscle and connective tissue fibers in the intima or the intimal aspect of the media, with their accompanying basement membranes. Any or all of the three elements may be involved. Since this process is seen in all age groups, it must be considered a normal reaction of the vein to mechanical stress. Thickenings in excess in number or size in any age group, however, constitute "increased endophlebohypertrophy," which may be considered a pathological finding.

Certain retrogressive changes occur in the vein which constitute phlebosclerosis. Some of these constantly occur in, and are more or less limited to, areas of endophlebohypertrophy. This type we shall call *endophlebosclerosis*. In order of their occurrence with advancing age, the changes constituting endophlebosclerosis are (1) basophilic staining (with hematoxylin and eosin), vacuolization and lacunar formation of the ground substance, (2) disruption and loss of elastic fibers, (3) loss of muscle cells and (4) replacement by collagenous connective tissue. Less constant severe changes are (1) fragmentation of the internal elastic lamella and (2) calcification. The ground substance histochemically shows increase, decrease or loss of stainable glycoprotein. The basement membranes around muscle and elastic tissue manifesting changes stain more sharply, less sharply or not at all with glycoprotein stain, and similar changes are found in the reticular component.

A possible interpretation of the changes in the ground substance and basement membranes may be offered, based on the work of Gersh and Catchpole. They consider an increase of stainability in the ground substance and basement membranes with McManus-Lillie-Hotchkiss technic as evidence of depolymerization of the glycoprotein constituent. In a later stage there is a decrease of stainability due to greater depolymerization and increased solution. Following their interpretation, we may say that in endophlebosclerosis there is a progressive depolymerization of the glycoproteins of the ground substance, and the glycoprotein component of the basement membranes around elastic and muscle tissue.

The frequency of occurrence of endophlebosclerosis varies from patient to patient within any age group, but it definitely increases with age. In the popliteal vein back pressure or stasis plays no role in the genesis of this type, nor is there any evidence that it increases the frequency of its occurrence.

The retrogressive changes occurring with advancing age and unrelated to endophlebohypertrophy are termed *senile phlebosclerosis*. These changes are both diffuse and focal. The diffuse change is an increase of connective tissue of the media (fig. 14 *C*). This becomes obvious in the fifth decade and is progressive thereafter. This is associated with a diminution of the number and hence with a loss of muscle cells in the media. The focal change is the deposition of calcium about the internal elastic lamella.

CHANGES IN THE ENDOTHELIUM

It was not possible to study adequately the changes in the endothelium in the series of human veins here presented. This was due to postmortem destruction. Five additional veins were injected with 4 per cent formaldehyde solution in situ, two under 5 to 10 mm. of mercury pressure. Even this did not enable us to see the endothelium uninterrupted. Where it was seen, it did not appear to be involved in the process of endophlebohypertrophy or sclerosis.

The endothelium was therefore studied in experimental endophlebohypertrophy. In a series of unpublished experiments Lev and Mackler [7] were able to produce endophlebohypertrophy and sclerosis in dogs by producing anastomosis between the proximal part of the femoral artery and the distal part of the femoral vein. In this series no endothelium proliferating downward in spots of endophlebohypertrophy was found (fig. 15 *A*). It is, however, uncertain whether the endothelium is destroyed in the process of endophlebohypertrophy.

ARTEFACTS

In the evaluation of our findings, postmortem changes had to be ruled out. The possible changes are (1) postmortem autolysis, (2) trauma incurred by contraction and stretching of the vein on removal, (3) formaldehyde artefacts and (4) overheating during paraffinization.

It was found that postmortem autolysis and overheating of fixed tissues result in changes of the ground substance similar to those described above. However, these changes are generalized and not localized to areas of endophlebohypertrophy. As a further proof that the changes in the ground substance are not due to postmortem autolysis, such changes were found in experimental endophlebohypertrophy, where tissue from the living dog was placed immediately into formaldehyde solution (fig. 15 *B*).

The role of formaldehyde in the production of artefacts was ruled out by the study of frozen-dried material. No remarkable difference in the staining properties of elastic, muscle and collagenous connective tissue was found between formaldehyde-fixed and frozen-dried material. However, differences were found in the morphological aspect of the ground substance. Part of the ground substance, especially that in the media and adventitia, was lost in formaldehyde fixation. That which remained had a tendency to move toward the basement membranes. Thus the basement membranes were accentuated, but there was less ground substance. In the regions where in formaldehyde-fixed material the ground substance appeared vacuolated with the hematoxylin and eosin stain and less stainable with the glycoprotein stain, in the frozen-dried material, the same process was evident but less marked (fig. 15 *C*). Therefore, the changes in the ground substance were not produced by formaldehyde fixation, although they were accentuated by it.

The role of traumatic stretching in the production of artefacts was evaluated by removing segments of vein without manipulation. The changes described above in all layers were repeated. We have, however, not ruled out the role of contraction in the production of artefacts.

7. Lev, M., and Mackler, S. A.: To be published.

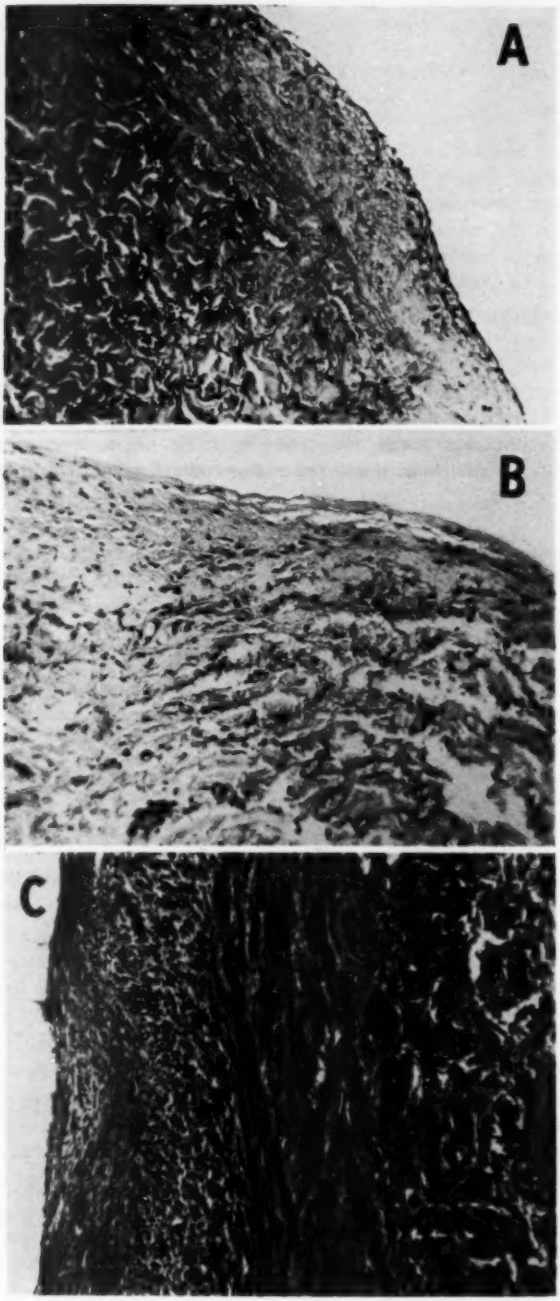

Fig. 15.—*A*, photomicrograph of a cross section of the femoral vein of a dog showing endophlebohypertrophy experimentally produced. There is no evidence of proliferation of endothelium. Hematoxylin and eosin stain; × 120.

B, photomicrograph of a cross section of the femoral vein of a dog showing changes in ground substance in endophlebohypertrophy, experimentally produced. Hematoxylin and eosin stain; × 120.

C, photomicrograph of a cross section of the popliteal vein, prepared by freezing and drying. Note the changes in ground substance in an area of endophlebohypertrophy. Hematoxylin and eosin stain; × 120.

THE COMMON DENOMINATOR IN MECHANICAL STRESS

We have seen that at the entries of tributaries and adjacent to the artery there are points of mechanical stress. In later reports it will be shown that adjacent bone or abnormal currents at certain points may also act in this manner. Histologically we have proved that there is a common denominator in the reaction of the vein in all these regions of stress—that is, endophlebohypertrophy. Is there, then, a common denominator in the type of stimulus producing this change? Although an exact answer is not yet forthcoming, there is suggestive evidence that there is.

At first glance it would seem that phenomena outside the vein can have little in common with intraluminal phenomena. A careful study, however, of the arterial effect on the vein reveals that at the point of maximal pressure either there is no change or atrophy of elastic and muscular tissue in all three coats occurs (fig. 16). Endophlebohypertrophy occurs just to one side or on both sides of this point of maximal pressure. This means that endophlebohypertrophy is not directly due

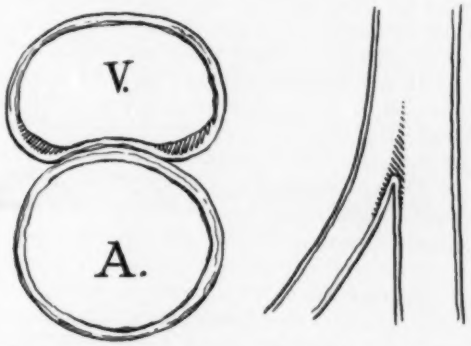

Fig. 16.—Diagram showing the possible mechanical effect of arterial contiguity and the entry of tributaries on the production of endophlebohypertrophy. *A*, artery. *V*, vein.

to arterial pressure on the wall of the vein. It would seem, then, that the effect produced is an indirect one. It is possible that this point of pressure, which is dynamic, because of arterial pulsation, results in a division of the current at this point, with the production of eddies on each side. This would then correspond to the effect of entering tributaries. In other words, lines of endophlebohypertrophy are "flow lines" or, in a sense, beginning septums in regions of divided or disturbed currents. Because of the low pressure in the veins as compared with that in the arteries, the veins are subject to pressure from both the inside and the outside, in contrast to arteries which react mostly to internal pressure. The common denominator in their mechanical effects is a disturbance in flow resulting in endophlebohypertrophy. Further and intensified mechanical stress results in endophlebosclerosis.

This does not imply that mechanical stress is the only factor in the production of endophlebosclerosis. We have not yet studied the role of the vasa vasorum. However, there is no evidence that cholesterol metabolism or inflammation plays any role in the genesis of endophlebosclerosis.

SUMMARY

The gross and the histological structure of the popliteal vein have been studied in all age groups.

A process of endophlebohypertrophy was found to begin from birth and to be especially localized at regions of mechanical stress. These areas are to one or both sides of the artery and at the entries of tributaries. This is considered to be a physiological process.

In the regions of endophlebohypertrophy, retrogressive changes progressively occur with advancing age. Such changes are included under the term endophlebosclerosis. They are differentiated from other retrogressive changes found in senile phlebosclerosis.

The possible common denominator in the mechanical factors responsible for the production of endophlebohypertrophy is a localized disturbance of blood flow.

ACUTE BACTERIAL ENDOCARDITIS DUE TO PSEUDOMONAS AERUGINOSA

Report of a Case

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ST. LOUIS

BACTERIA of the family Pseudomonadaceae are generally considered to be saprophytic in the skin and the gastrointestinal tract of man,[1] but one species, Pseudomonas aeruginosa (Bacillus pyocyaneus), has been proved to be pathogenic for man under certain conditions. These organisms, widely distributed in nature, are found in water and soil and in the fecal contents of man and animals, and they impart a fluorescent green, yellowish or blue pigment to the exudates which they produce. Hyman and Edelman,[2] Scott[3] and Carroll, Allen and Doubly [4] have shown that this organism may also invade the urinary tract and cause bacteremia following urological procedures.

Ps. aeruginosa has been reported to have caused infantile diarrheas of epidemic proportions,[5] and also meningitis has occurred in consequence of contamination during spinal anesthesia.[6] Otitis media, mastoiditis and primary infections of the liver and the spleen have been shown to be due to this bacterium.

The case herein reported is unique in that, first, it was characterized by involvement of the aortic and mitral valves, second, the primary site appears to have been in the lung and, third, the septic course was unaffected by the newer antibiotics.

HISTORY OF CASE

A 53 year old white woman entered the hospital on March 24, 1950. She had been in excellent health until three weeks before admission when she contracted "flu," at which time fever, headache, pain across the shoulders, weakness, slight pain in the arms and anorexia were noted. At night she became dyspneic, and a slight degree of hemoptysis occurred some mornings on awakening. The patient had been bedridden since the onset of her illness, and during this time edema of the lower extremities developed.

Physical examination revealed an obese, listless white woman who was perspiring profusely and had a flushed facies. The respirations were 36 per minute, the pulse rate 110 per minute, the temperature 105.5 F. and the blood pressure 80/50.

From the Departments of Pathology and Medicine, St. Louis University School of Medicine.

1. Fraenkel, E.: Ztschr. f. Hyg. u. Infektionskrankh. **95**:125-134, 1922.

2. Hyman, A., and Edelman, L. J. Urology **28**:173-198, 1932.

3. Scott, W. W.: J. Urology **21**:527-566, 1929.

4. Carroll, G.; Allen, H. N., and Doubly, E. K.: Study of Bacillary Infections of Urinary Tract, J. A. M. A. **135**:683-686 (Nov. 15) 1947.

5. Hunter, C. A., and Ensign, P. R.: Am. J. Pub. Health **37**:1166-1169, 1947.

6 Vuylsteke, C. A.: Brit. M. J. **1**:179-180, 1947.

Fine rales were heard throughout both lung fields, with increased breath sounds and dulness over the lower lobe of the right lung. Tenderness on palpation of the right upper quadrant of the abdomen was elicited, with no muscle guard. Anasarca with pronounced pitting edema of the thighs and legs was present. The urine was observed to be emerald green on several occasions. Hematuria and pyuria occurred, and a positive culture of Ps. aeruginosa was obtained from the urine. The hemogram was: red blood cells, 2.6 million; hemoglobin, 9 Gm.; white cells, 14,800 up to 33,700, with a moderate shift to the left on the Schilling count. The Kahn test, made on both the blood and the spinal fluid, was negative. The blood nonprotein nitrogen ranged from 48 to 75 mg. per 100 cc. Blood culture was positive for Ps. aeruginosa on four different days. Feces and material from the throat also gave growths of this organism. The spinal fluid pressure was 300 mm. of water, and the fluid contained 287 polymorphonuclear granulocytes per cubic millimeter. Spinal fluid proteins were 72 mg. per 100 cc.; sugar, 67 mg., and chlorides, 754 mg. per 100 cc. A smear of the spinal fluid revealed gram-negative, pleomorphic rods, but the culture did not show growth. Serum agglutination tests for the typhoid group were positive at a dilution of 1 to 160; all the others were negative. An electrocardiogram showed a heart rate of 180 per minute, auricular tachycardia and myocardial damage. A roentgenogram of the chest revealed the base of the right lung to be infiltrated, with obliteration of the right costophrenic sinus.

During the patient's six days in the hospital a marked febrile course was maintained, with increased manifestations of lethargy and somnolence. She experienced sharp pains in her right shoulder and became quite edematous. Therapeutic doses of streptomycin, chloromycetin,* aureomycin and penicillin were given throughout her hospital stay, but the course of illness was unaltered. On the afternoon of the sixth hospital day the patient's blood pressure dropped, her pulse became feeble and she quietly succumbed.

PATHOLOGICAL OBSERVATIONS

The heart weighed 200 Gm. and was normal in size and shape but flabby. When the heart was opened, vegetations adherent to the aortic and mitral valves were seen. Attached to the posterior and lateral cusps of the aortic valve were apron-like sheets of reddish tan vegetations, measuring 1.5 by 1 by 0.3 cm. and 1 by 1.3 by 0.6 cm. (fig. 1). These vegetations were quite soft and were easily detached from the valves. In the lateral cusp of the aortic valve there was a rounded patent defect, 4 mm. in diameter, which was considered to be due to the inflammatory endocarditic process and which caused a partial valvular incompetency. The free edges and the commissures of the valves were normal and showed no evidence of previous inflammation.

About five or six vegetations, 1 mm. in diameter, were present on the leaflets of the mitral valve and the chordae tendineae. The valve measurements and the coronary arteries, the epicardium and the myocardium were entirely normal. Histological examination of the vegetations showed a single layer of intact endocardial cells covering a homogeneous pink fibrinous stroma which contained small numbers of acute and chronic inflammatory cells. Many rather symmetrical clumps of gram-negative, slightly pleomorphic short bacilli were uniformly distributed in the stroma (fig. 2). A few plasma cells and mononuclear cells were present in the fatty connective tissue of the epicardium. Smears and cultures of the valvular vegetations revealed the organisms to be Ps. aeruginosa.

The right lung weighed 410 Gm. and contained an abscess 2 cm. in diameter in the lower lobe, situated 1 cm. from the diaphragmatic edge near the median edge of the lobe. This abscess was filled with a yellowish green, purulent material and did not communicate with a bronchus. The left lung weighed 310 Gm. and was not remarkable. Microscopically, the abscess was lined by several layers of metaplastic squamous epithelial cells, and surrounding this were acute and chronic inflammatory cells and some granulation and fibrous tissue. Emphysema of both lungs was evident.

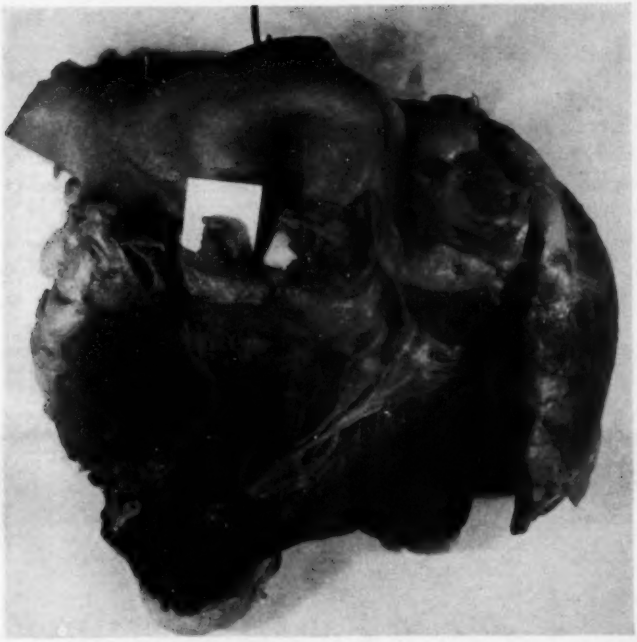

Fig. 1.—Vegetations may be seen on the posterior and lateral cusps of the aortic valve, and a defect in the cusp on the right (indicated by protruding cotton).

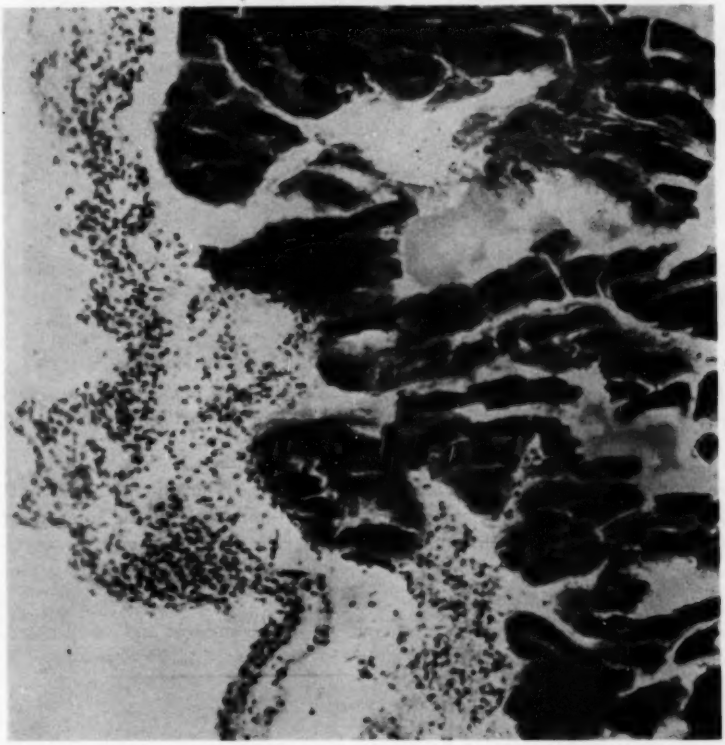

Fig. 2.—Section of the aortic vegetation, which shows clumps and groups of bacteria with some round cell infiltration; × 430.

The liver weighed 2,540 Gm., appeared enlarged and contained many abscesses, the largest of which was 6 cm. in diameter and was located on the superior surface in the region of the falciform ligament. This abscess was composed of a dark greenish yellow necrotic material, the bulk of which was seen to be contained within the liver, and a portion of the right diaphragm formed the superior boundary. The numerous smaller metastatic abscesses all were filled with a greenish yellow exudate. Many mycotic thrombophlebitic lesions were present. The unaffected

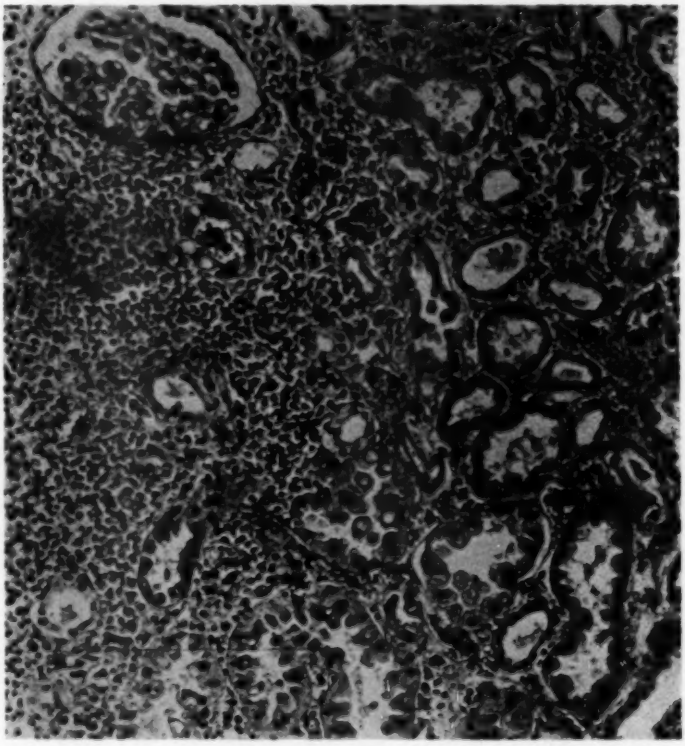

Fig. 3.—The interstitial tissue of the kidney shows a diffuse and moderately heavy infiltration of plasma cells, mononuclear cells and a few polymorphonuclear granulocytes; × 430.

parenchyma was yellowish tan and had a greasy appearance. Microscopically, there were moderate fatty infiltration and metamorphosis. The abscesses were of the acute, purulent type and contained cellular debris and fibrinous necrotic material, as well as some acute and chronic inflammatory cells. The limiting wall was composed of granulation tissue, but the adjacent hepatic cells revealed a lytic type of necrosis. Sparse focal areas of fibrosis were present.

The spleen weighed 610 Gm. and contained fresh infarcts and small abscesses similar to those seen in the liver.

The kidneys had a total weight of 490 Gm. and revealed several small (1 cm.) greenish yellow abscesses subcapsularly as well as deeper in the parenchyma. The tissue of the surface of the kidney was reddish brown and contained small petechiae. There was an unusually diffuse and massive interstitial infiltration of plasma cells, lymphocytes and a few neutrophils, with no evidence of involvement of the glomeruli or the tubules (fig. 3). This type of reaction was described by Councilman [7] and was labeled acute interstitial nephritis, in contrast to true suppurative nephritis. The pelvis and peripelvic fat were not infiltrated by these inflammatory cells. A slight degree of arteriosclerosis was seen in the larger vessels. The abscesses microscopically resembled those previously described. A few hyaline and cellular casts were noted in the collecting tubules.

The cerebrospinal fluid removed post mortem was clear, and the culture revealed no growth. The brain and meninges were grossly normal, but on microscopic examination diffuse chronic meningitis was seen. This was manifested in thickening and fibrosis of the meninges and accumulations of plasma cells, mononuclear cells and polymorphonuclear granulocytes (fig. 4). Perivascular cuffing and infiltration of peripheral portions of brain tissue by acute and chronic inflammatory cells were noted. Moderate numbers of small focal embolic inflammatory areas were seen throughout the brain.

The anatomic diagnoses were as follows: acute bacterial endocarditis of the aortic and mitral valves due to Ps. aeruginosa; septicemia; abscess of the lower lobe of the right lung with squamous metaplasia; multiple abscesses of the liver, the kidneys and the spleen; infarcts of the spleen and the kidneys; focal embolic encephalitis; subacute meningitis; acute interstitial nephritis; chronic intramural enteritis; myometritis; marked myeloid hyperplasia; fatty metamorphosis and infiltration of the liver; chronic interstitial pancreatitis; periportal fibrosis of the liver; focal atelectasis of the lungs; emphysema of the lungs; accessory spleen; edema of the lower extremities; healed bilateral pleurisy; dental caries.

BACTERIOLOGIC REPORT

Antemortem cultures of blood, urine, feces and material from a throat swab were positive for B. pyocyaneus: The bacteria were gram-negative, non-spore-forming, motile, pleomorphic rods which liquefied gelatin and produced green pigment in litmus milk. Colonies grew on blood agar, heart infusion broth and agar, Loeffler's medium, indole agar and gelatin. These colonies were numerous, circumscribed and greenish blue. None of the sugars were fermented. A smear of the cerebrospinal fluid late in the illness was positive for bacteria, but the cultures were negative. This failure of the culture to show growth was thought to be due to attenuation of the bacteria from heavy doses of chemotherapeutic agents which had been used throughout the patient's stay in the hospital.

Postmortem cultures of blood, urine, feces, cerebrospinal fluid, lung abscess material and kidney were also positive for Ps. aeruginosa (fig. 5).

COMMENT

In a review of the literature concerning cases comparable to this one, several interesting features were noted (table). The liver was involved with abscesses in

7. Councilman, W. T.: M. & S. Rep. City Hosp., Boston, 1897, p. 174.

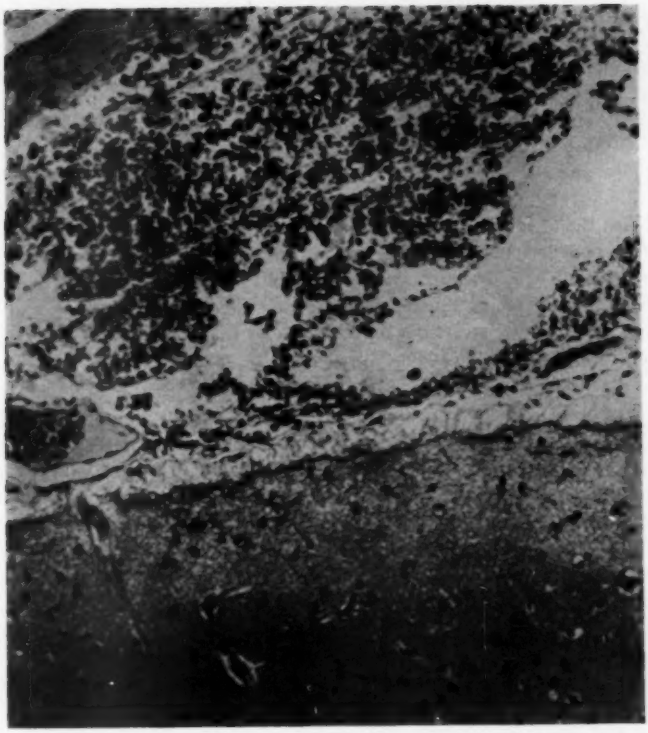

Fig. 4.—The meninges are thickened by accumulations of plasma cells, mononuclears and a few neutrophils; × 430. Some blood has escaped into the subarachnoid space.

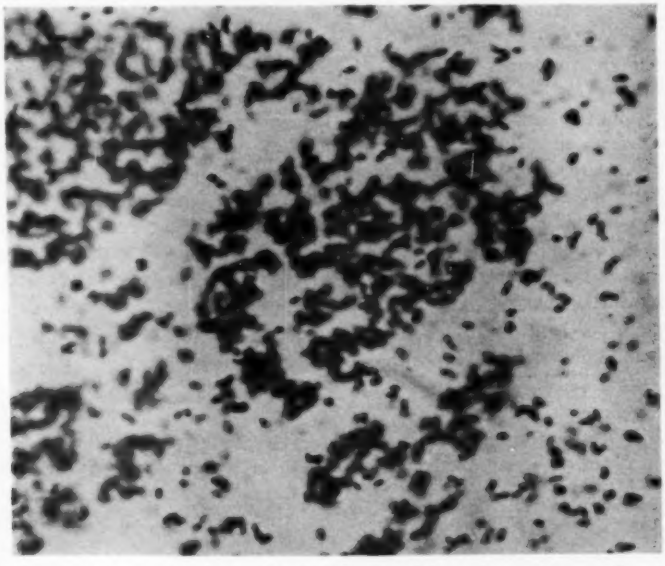

Fig. 5.—The short, somewhat pleomorphic bacilli cultured from the aortic vegetation; × 1,000.

one other case.[8] There was previous damage of the heart valve in about one third of the cases, and 40 per cent showed some involvement of other portions of the vascular tree. There appeared to be a predilection for the mitral valve, for in seven of the 10 cases this valve was involved

In our consideration of the present case several unusual points arise. In the previously reported cases the portals of entry were the skin, the gastrointestinal tract and intrathecal contamination. However, in this case the pulmonary abscess was considered to be the primary focus of infection because of the age of the lesion, indicated by squamous metaplasia and fibrosis. The patient's history substantiated

Reported Cases of Bacterial Endocarditis Caused by Pseudomonas Aeruginosa

Author	Age, Yr.	Sex	Valve Involved	Previous Damage of Valve	Probable Portal of Entry	Other Organs Involved
1. Blum, S.: Zentralbl. f. Bakt. **25**: 113-116, 1899	2½ mo.	M	Mitral	No reference	No reference	No reference
2. De la Camp: Charité-Ann. **28**: 92-111, 1903.	51	F	Mitral	No reference	Skin	Skin, intestines, spleen
3. Rolly: München. med. Wchnschr. **53**: 1399-1404, 1906	28	F	Mitral	Yes	Gastrointestinal tract	Meninges, spleen, kidneys, arteries
4. Thayer, W. S.: Johns Hopkins Hosp. Reports **22**: 73-74, 1926	42	F	Mitral	No reference	Gastrointestinal tract	Ovaries, intestine, peritoneum
5. Büngeler, W.: Frankfurt. Ztschr. f. Path. **35**: 428-435, 1927	65	M	Mitral	Yes	Contaminated intravenous injection	Spleen
6. Kearns, J. J.: Arch. Path. **21**: 839-843 (June) 1936	35	M	Aortic and mitral	No	Skin	Aorta, pulmonary artery
7. Fish, G. W.; Hand, M. M., and Klein, W. F., Jr.: Am. J. Path. **13**: 121-128, 1937..	71	M	Aortic	No reference	Genitourinary tract	Kidneys, aorta
8. Moragues, V., and Anderson, W. A. D.: Ann. Int. Med. **19**: 146-154, 1943	60	M	Mitral	Yes	Genitourinary tract	Kidneys, lungs, prostate, spleen, meninges and arteries
9. Stanley, M. M.: Am. J. Med. **2**: 253-277 and 347-367, 1947	45	M	Tricuspid	Yes	No reference	Lung
10. DeMuth, Jr.,[8] and Rawson	40	M	Aortic	No	Gastrointestinal tract	Liver, kidney
11. Coller and Dyer	53	F	Aortic and mitral	No	Lung	Meninges, liver, spleen, kidneys, brain, intestines, uterus, peritoneum

the idea of the lung as the portal of entry. The acute interstitial nephritis, a condition described by Councilman years ago, is of interest because of its rarity at the present time.

SUMMARY

A case of acute bacterial endocarditis due to Pseudomonas aeruginosa is reported, in which there were widespread embolic abscesses of the viscera and the brain, meningitis and acute interstitial nephritis.

Ten previously reported cases are compared with the case presented.

It is believed that this is the first case in which the respiratory tract was the portal of entry.

8. DeMuth, W. E., Jr., and Rawson, A. J.: Am. J. M. Sc. **216**:195-202, 1948.

THE SYMPATHETIC GANGLIONS OF HYPERTENSIVE PATIENTS

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IN RECENT years the sympathetic nervous system has attracted the research interests of the neuroanatomist, the physiologist, the pharmacologist and members of the clinical specialties. Such investigations are constantly unraveling the complicated fiber pathways and functions of this dispersed portion of the nervous system. This increased knowledge has provided a spring board for diagnostic procedures and rational attacks on the sympathetic nervous system in the surgical relief of persons suffering from pain, tumors and a variety of vasomotor disturbances.

Studies of particular interest to me were those investigations which were made in an attempt to correlate morphological changes in human sympathetic ganglion cells with known disease states.

The histopathological studies appearing in the literature have been carried out either on ganglions obtained at autopsy or on surgically removed ganglions. Unfortunately these investigations have yielded diverse and contradictory results.

Staemmler [1] observed both cytoplasmic and nuclear changes in the neurons of the cervical sympathetic ganglions, which he attributed to antemortem infectious disease and vasomotor disturbances. He found the ganglions to be hyperemic and infiltrated with mononuclear leukocytes; the cytoplasm of the nerve cells was swollen and stained less intensely. The nuclei were often enlarged and eccentric, and in some the nucleoli had disappeared. These cytologic changes led to cell atrophy and eventual necrosis. Kuntz,[2] after studying the cervical and lumbar sympathetic ganglions in 30 cases, concluded that hyperplasia of the interstitial connective tissue of the ganglion is the result of chronic inflammation, while melanin deposition and the presence of hyperchromatic ganglion cells are indicative of functional depression. Although chromidial content, moderate melanin pigment and occasional degenerative cellular changes were acknowledged as age variables, Kuntz [3] concluded that definite disease change is indicated by marked or excessive chromidial loss, melanosis, neuronophagia and proliferation of the capsular nuclei. Some of the above neuronal changes have recently been produced in rabbits by experimental feeding of cholesterol (Kuntz and Sulkin [4]).

From the Department of Anatomy, Hahnemann Medical College and Hospital.

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1. Staemmler, M.: Deutsche med. Wchnschr. **50**:457-459, 1924.

2. Kuntz, A.: Sympathetic Ganglions Removed Surgically: Histopathologic Study, Arch. Surg. **28**:920-935 (May) 1934.

3. Kuntz, A.: Am. J. Path. **14**:783-795, 1938; Biol. Symposia **11**:101-117, 1945.

4. Kuntz, A., and Sulkin, N. M.: Lesions Induced in Rabbits by Cholesterol Feeding, with Special Reference to Their Origin, Arch. Path. **47**:248-260 (March) 1949.

In contrast to the above findings, Clark [5] found no correlated histopathological changes in the cervical sympathetic ganglions in a series of 7 cases of angina pectoris. Craig and Kernohan [6] compared 208 cases of surgically removed ganglions with 40 nonoperative autopsy control cases. Specimens of cervical, thoracic and lumbar ganglions were included in the series studied. These authors concluded that pigmentation, cellular infiltrations, capsular proliferation and vascular changes are not indicative of disease but are to be explained on the basis of advancing age. In a recent study Bergmann, Harmon, Pick and Wertheim [7] have compared the sympathetic ganglions surgically removed from 24 persons suffering from vascular disease with 41 ganglions obtained at autopsy. They were able to demonstrate the cytological changes described by the previous investigators. Ganglions from patients with hypertensive cardiovascular disease also demonstrated a thickening of the walls of the intraganglionic venules. These authors concluded that a characteristic histological picture of sympathetic ganglions which is indicative of vascular disease does not exist.

The foregoing controversial conclusions regarding the presence or the absence of specific lesions in the sympathetic ganglions in certain disease states seemed to warrant further investigation. Thus the present study was undertaken to determine the validity of consistent structural changes which might serve as criteria indicative of the hypertensive state in man.

MATERIAL AND METHODS

A total of 44 thoracic and lumbar sympathetic trunk ganglions surgically removed from 17 persons (30 to 59 years of age) who suffered from arterial hypertension are included in the present report. Twenty-one human control ganglions were obtained at 18 autopsies from one to six hours after death (the persons represented were 29 to 80 years of age).

After appropriate fixation (formaldehyde solution U. S. P., ferric chloride, potassium bichromate, Orth's fluid, mercury bichloride, chloral hydrate, chilled 80 per cent alcohol) serial paraffin sections of the ganglions were stained by one or more of the following technics: Masson, Bodian, benzidine, erythrosin-toluidine blue, silver nitrate, chromaffin or alkaline phosphatase.

RESULTS

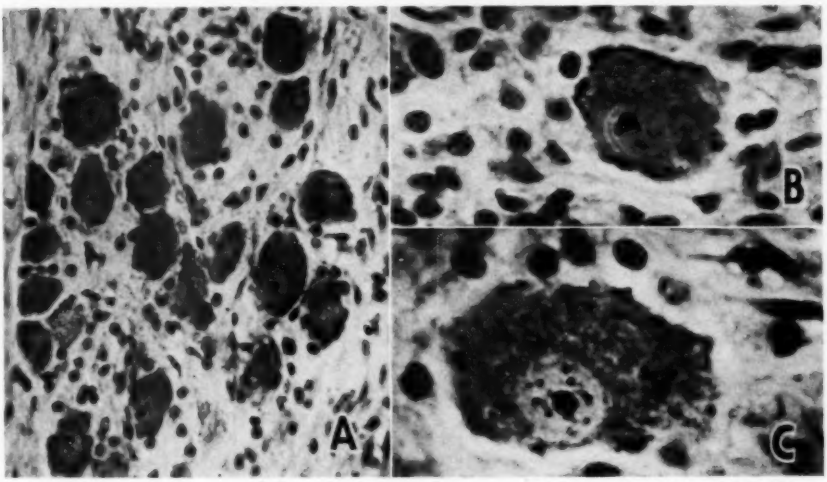
Histological examination of serial sections of the ganglions of patients with arterial hypertension demonstrated both large and small areas of definite cellular and intercellular change; yet similar intraganglionic alterations could be demonstrated in the control sympathetic ganglions from persons who did not have arterial hypertension. Such structural alterations were most impressive, for in the absence of adequate control specimens they might easily be construed as specific neuronal changes indicative of the hypertensive state.

Thus, one not infrequently observes sympathetic ganglion cells in what appears to be an advanced stage of chromatolysis involving an extensive segment of a ganglion. Such pale cells seem devoid of chromidial substance, and one may find only an occasional nerve cell with a semblance of the normal quantity and distribution of cytoplasmic chromatin (Ingersoll [8] and Kuntz [9]). The chromatolytic

5. Clark, S. L.: J. Lab. & Clin. Med. **13**:101-109, 1927.
6. Craig, W. M., and Kernohan, J. W.: Surg., Gynec. & Obst. **56**:767-778, 1933.
7. Bergmann, L. L.; Harmon, P. J., and Wertheim, H. M.: Surgery **24**:695-702, 1947.
8. Ingersoll, E. H.: J. Comp. Neurol. **59**:267-283, 1934.
9. Kuntz, A.: The Autonomic Nervous System, Philadelphia, Lea & Febiger, 1945.

cells may even resemble the severely altered nerve cells seen in "axon reaction," i.e., following section of the axon of a nerve cell. However, if one examines adjacent serial sections one finds that most of the cells appear to be normal. This has been my experience in both the controls and the ganglions from persons with arterial hypertension.

The nuclear chromatin is commonly believed to replenish the depleted cytoplasmic chromatin in fatigued and functionally depressed nerve cells (Kuntz). One is not surprised, therefore, to find ganglionic areas in which many of the cells demonstrated hyperchromatic nuclei (fig. 1 *A*). In such areas one can see the intranuclear masses of chromatin closely opposed to the nucleolus, and, as in figure 1 *B*, they often appear to be budding off the nucleolus. Ten to fifteen discrete clumps of nuclear chromatin are not unusual in the ganglion cells of such an area. These hyperchromatic nuclei (fig. 1 *C*) stand out in bold contrast when compared



1.—*A*, hyperchromatic nuclei of sympathetic ganglion cells. Note one to three extra chromatin masses in addition to a discrete nucleolus. (Lumbar ganglion of a woman aged 53 years with hypertensive cardiovascular disease; erythrosin-toluidine blue; × 250.)

B, hyperchromatic nucleus of a ganglion cell. Chromatin bodies appear to be budding off the nucleolus. (Same specimen as *A*; × 400.)

C, sympathetic ganglion cell with nucleus containing 12 discrete chromatin bodies dispersed about the nucleolus. (Same specimen as *A*; × 550.)

with the usual vesicular nucleus of the nerve cell which contains a single prominent nucleolus. The larger nuclear masses of chromatin appear to undergo fragmentation to form smaller, dustlike particles. The latter are more peripherally located within the nucleus, adjacent to the nuclear membrane.

The position and the character of these chromatin particles indicate that they may be transported through the nuclear membrane to replenish the cytoplasmic chromatin.

Although ganglionic areas with hyperchromatic nuclei (fig. 1) were observed best in the sympathetic ganglions of persons suffering from arterial hypertension, they should not be considered as pathognomonic for cardiovascular disease.

These two observations, namely, extensive cellular chromatolysis in one area and hyperchromatism of nuclei in another, lead one to assume that there is a great variation in the amount of cytoplasmic chromatin in the normal sympathetic cells of any given ganglion and that the cytoplasm can readily be replenished from the cell's own nucleus. Thus the often quoted reference to chromatolytic changes in the sympathetic ganglion cells is subject to wide individual interpretations and undoubtedly accounts for many of the divergent opinions expressed in the literature. My associates and I have exercised the greatest of caution lest we designate the normal functional depletion of the neuron's cytoplasmic chromatin as a criterion of cell degeneration or as characteristic of the hypertensive state. Such cells have normal cell contours and are probably capable of rapidly restoring their full complement of chromidial substances. In our opinion they are still within the realm

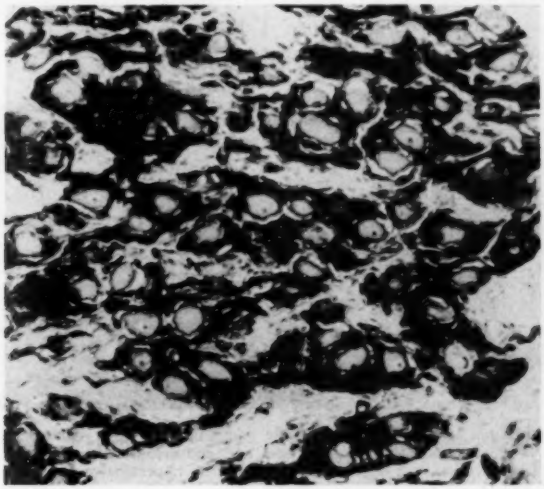


Fig. 2.—Capsular nuclei (stained black) of sympathetic ganglion cells demonstrating their content of the enzyme alkaline phosphatase. (Lumbar ganglion of a woman aged 30 years, with hypertensive cardiovascular disease; Gomori technic; × 150.)

of normal cells, but to deduce their antemortem functional capacity from postmortem fixed and stained preparations in the light of present knowledge is most unwarranted.

We have also observed neurons with eccentric and multiple nuclei in both the controls and the ganglions from patients with arterial hypertension. Melanin is deposited in variable amounts in the ganglion cells of persons of all ages. We believe such variables to be dubious criteria if used in designating a specific hypertensive state in the sympathetic ganglions of man.

Particular histological attention was directed to the capsular and interstitial nuclei in the ganglions of patients with arterial hypertension. One is often amazed at the number of small, round, closely packed nuclei seen in sections of sympathetic ganglions. The nuclei are so abundant at times as to mask the basic appearance

of the interstitial tissue of the ganglion. It is difficult in such instances to distinguish between capsular, neurilemal, and interstitial nuclei. One has observed such nuclear hyperplasia most commonly in sympathetic ganglions from carcinomatous persons, but such areas were also found in the sympathetic ganglions of the controls and the persons with arterial hypertension. Proliferation of the capsular nuclei alone often presents a striking picture, especially when observed in sections prepared by the alkaline phosphatase technic (fig. 2).

In such preparations one sees only the nuclei stained black (because of their enzyme content) while the ganglion cells, the nerve fibers and the interstitial tissue provide a neutral, lightly stained background. Although such a histochemical appearance of the capsular nuclei provides information as to their enzyme content, the exact role of these conspicuous nuclei has never been conclusively demonstrated.

The numbers of capsular and interstitial nuclei are extremely variable in apparently normal sympathetic ganglions. I do not believe they provide a reliable index indicative of the antemortem hypertensive state.

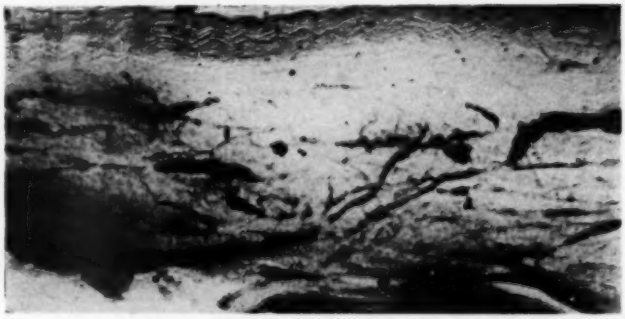


Fig. 3.—Longitudinal section of a sympathetic ganglion showing its sinusoidal network and abundant blood supply. Note that small veins are continuous with dilated ampullae and sinusoids. (Lumbar ganglion of a man aged 59 years, with hypertensive cardiovascular disease; benzidine stain; × 32.)

In a hypertensive state one might logically expect to note marked changes in the walls of the ganglionic arterioles. Such changes are to be found, but in no greater incidence than in the ganglions of persons of similar age groups who do not have arterial hypertension. The abundant vascular supply of the sympathetic ganglions and the appearance with age of a dilated sinusoidal network are shown in figure 3. As pointed out in previous communications (Truex[10]) the abundant blood supply of the sympathetic ganglions may account for the modest amount of degenerative changes observed with age, as compared with the sensory ganglions of man. For a detailed description of the blood supply of autonomic ganglions the reader is referred to the work of Bergmann and co-workers.[11]

10. Truex, R. C.: Am. J. Path. **16**:255-268, 1940. Truex, R. C., and Zwemer, R. L.: True Fatty Degeneration in Sensory Neurons of the Aged, Arch. Neurol. & Psychiat. **48**:988-995 (Dec.) 1942.

11. Bergmann, L.: Anat. Rec. **85**:117-133, 1943. Bergmann and others.[7]

COMMENT

The present observations on the sympathetic ganglions of patients with arterial hypertension are in agreement with those reported by Bergmann and associates.[7] It was not possible in the study here reported to demonstrate specific cellular changes or ganglionic lesions which could be interpreted as characteristic of this disease state. One feels confident that the usual artefacts and postmortem autolysis of cells were eliminated, since the specimens studied were all rapidly fixed after surgical excision. There is no doubt of the validity of ganglion cell chromatolysis, melanosis and nuclear proliferation. However, the normal range of these cell alterations is exceedingly great in the sympathetic ganglions of persons of all ages.

It should be remembered that error is often inherent in histological technics. Such errors must always be foremost in mind before drawing extensive conclusions from specimens stained by the various methods used to demonstrate cytoplasmic chromatin of nerve cells. Neuronal chromatolysis has proved a most valuable neurological method with which one can demonstrate the cell effects resulting from disease and experimental lesions. However, what appears to be neuronal chromatolysis can also be the result of prolonged decolorization in the process of preparing the microscopic sections when certain Nissl methods are used.

SUMMARY AND CONCLUSIONS

Histological study of 44 thoracic and lumbar sympathetic ganglions surgically removed from 17 patients with arterial hypertension failed to demonstrate any consistent lesions or ganglion cell changes which could be interpreted as indicative of the hypertensive state.

In sympathetic ganglions from such patients one can observe areas of neuronal chromatolysis, ganglion cells with hyperchromatic nuclei, changes in vascular pattern, proliferation of capsular nuclei and variable amounts of cellular pigmentation. However, similar neuronal changes are found also in ganglions removed from control patients of similar ages who do not have arterial hypertension. The cell changes cited manifest a wide range of variation in apparently normal individuals.

One may not designate structural alterations in a sympathetic ganglion as characteristic of the hypertensive state in the absence of more advanced, discrete and reliable neuropathological findings. To propose a diagnosis of cardiovascular disease on the basis of the above variable criteria alone is a questionable procedure.

THE POSTNATAL STRUCTURAL CHANGES IN THE INTRAPULMONARY ARTERIES AND ARTERIOLES

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STUDY of the structure of the pulmonary vascular tree in certain congenital cardiac malformations and in other conditions makes it imperative that anatomic base lines be established. The structure of some of the elements of the pulmonary vascular tree in the fetus varies considerably from that in the adult. Between these two extremes there are gradations. All these are to be recognized if a correct interpretation is to be made as to the normality or the abnormality of the pulmonary arterial vessels in a given case.

The study to be reported is that of the microscopic appearance of the normal pulmonary arteries at all ages with particular emphasis on the changes which occur from fetal life to the time when adult characteristics become established.

Sections of lungs obtained at necropsies from fetuses and persons up to the age of 73 years were examined microscopically. At least three sections of each pair of lungs were studied, and in most instances five sections were examined.

All sections were from tissue which had been fixed in solution of formaldehyde U. S. P. for at least 24 hours. They were prepared by the standard paraffin technic and stained with hematoxylin and eosin. Sections from representative blocks were also stained with Verhoeff's elastic tissue stain and counterstained with Van Gieson's connective tissue stain.

The nomenclature suggested by Brenner [1] was employed in this work. Brenner's classification considers three types of intrapulmonary arteries. These are (1) elastic arteries, (2) muscular arteries and (3) arterioles.

The elastic arteries are the largest arteries and in the lung have diameters of about 1 mm. and larger. Microscopically, those elastic arteries that have acquired adult characteristics show an endothelial lining that lies close to a thick internal elastic membrane. The media is composed predominantly of elastic laminas. Intervening smooth muscle fibers are moderately numerous. Collagenous fibers are infrequent. The larger the vessel the more numerous the elastic laminas.

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Abridgment of a portion of a thesis submitted by Dr. Civin to the Faculty of the Graduate School of the University of Minnesota in partial fulfilment of the requirements for the degree of Master of Science in Pathology.

1. Brenner, O.: Pathology of Vessels of Pulmonary Circulation, Arch. Int. Med. **56**:211 (Aug.); 457 (Sept.); 724 (Oct.); 976 (Nov.); 1189 (Dec.) 1935.

The latter are long and usually continuous around the wall circumferentially, but may appear interrupted. The adventitia varies in thickness and is composed of thick collagenous fibers and a few cells.

The next type of intrapulmonary artery is that of the muscular arteries. These vessels merge imperceptibly with the elastic arteries on the one hand and with the arterioles on the other. The muscular arteries are, in general, smaller than the elastic arteries, have smooth muscle as their preponderant medical element and have a dense adventitial coat. In muscular arteries with adult characteristics elastic fibers usually are seen only in the forms of the internal and external elastic laminas, although a few isolated strands may be found normally in the media.

The arterioles are the vessels with diameters of about 0.1 mm. (100 microns) or less. They lie in close proximity to the alveolar spaces. The arteriole consists of a layer of endothelium surrounded by collagen and at times by a single elastic lamina. The adventitia is thin. The thickness of the wall measures less than 5 per cent of the diameter of the vessel lumen. Arterioles are difficult to distinguish from venules. The only way definitely to establish a vessel as a venule or as an arteriole is to see its junction with the parent venous or arterial stem.

The capillaries are the major constituents of the alveolar septums. The veins that are more than 0.25 mm. in diameter have a media that is composed of irregular elastic fibers and a few smooth muscle cells. A thick adventitia often merges imperceptibly with the media.

OBSERVATIONS

During the last trimester of pregnancy the lungs of the fetus present a polygonally lobular pattern. Within the lobule there are usually from one to three bronchioles. The epithelium of these bronchioles consists of ciliated columnar cells possessing basal nuclei. The cells rest on a basement membrane. Peripheral to this is a layer of loose, at times myxomatous, relatively acellular connective tissue. Adjacent to each bronchiole is a thick connective tissue matrix containing an artery that is about half the diameter of the bronchiole. This is a muscular artery. Small veins accompany the artery. Together the bronchiole, the associated vessels and the supporting tissue make an anatomic complex. Surrounding these intralobular complexes are groups of alveoli lined by cuboidal cells and separated by loose connective tissue. The lobules are bounded by interlobular septums. At one or more corners in the interlobular septums there is a bronchus lined by pseudostratified columnar epithelium, also resting on a basement membrane. Beneath this is a layer of loose collagenous tissue. Peripheral to this are rings of cartilage surrounded by dense connective tissue containing mucous glands. Outside the bronchial wall is an elastic artery which usually is about half the size of the bronchus it accompanies. The bronchi and the arteries located in the interlobular septums are about twice the size of the bronchioles and muscular arteries within the lobule.

The time of disappearance of the obviously microscopically lobular character of the lung varies. In one patient, who lived eight minutes after birth, the lobular structure of the lung was not apparent. In other cases the lobular character was retained for several months after birth.

The structural changes occurring in the intrapulmonary arterial vessels from fetal life to maturity will be described under the headings of their various anatomic classes.

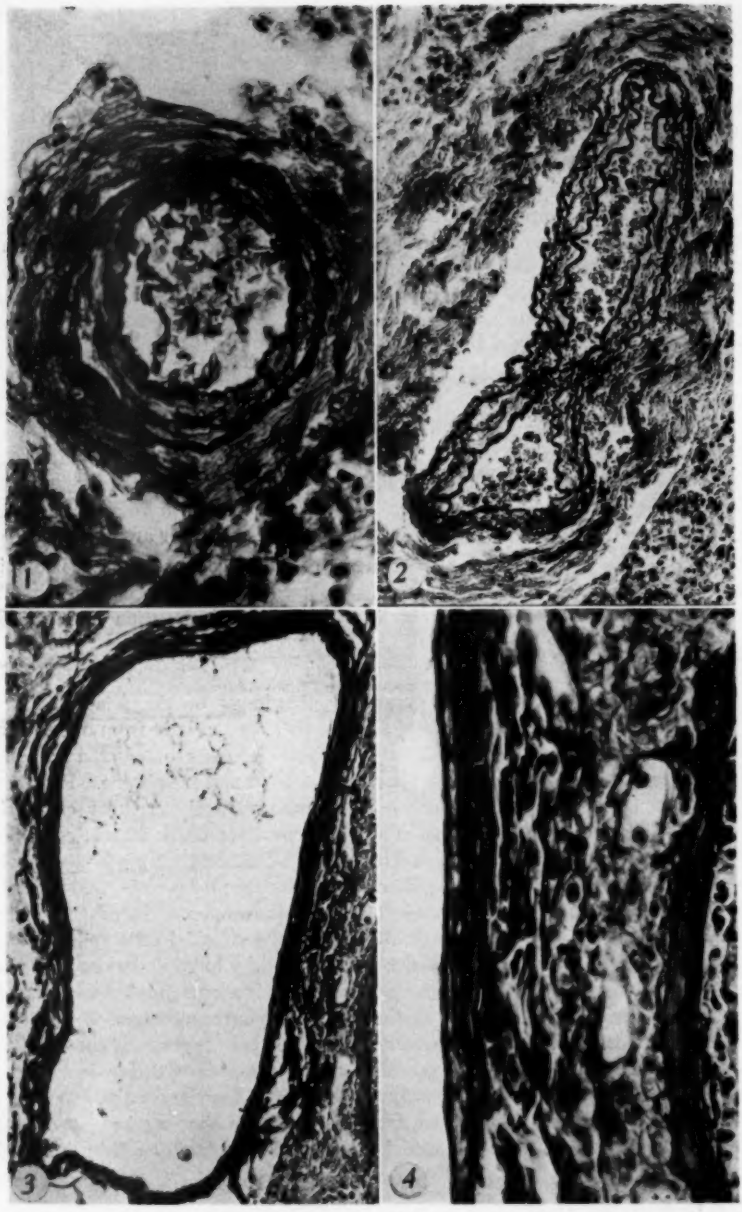

Fig. 1.—An elastic artery of the lung of a stillborn fetus. The internal and external elastic laminas are well defined. A relatively small number of irregular strands of elastic tissue are present within the media. (Verhoeff's elastic tissue stain counterstained with Van Gieson's connective tissue stain; × 450.)

Fig. 2.—An elastic artery of the lung of an infant 7 days old. The internal elastic lamina is prominent, and irregular strands of elastic tissue are present in the media. (Verhoeff's elastic tissue stain counterstained with Van Gieson's connective tissue stain; × 170.)

Fig. 3.—An elastic artery of the lung of an infant 6 months old. The internal and external elastic laminas are well demarcated. Elastic fibers are present within the media. (Verhoeff's elastic tissue stain counterstained with Van Gieson's connective tissue stain; × 200.) A higher magnification of this artery is illustrated in figure 4.

Fig. 4.—Higher magnification of the elastic pulmonary artery illustrated in figure 3. (Verhoeff's elastic tissue stain counterstained with Van Gieson's connective tissue stain; × 450.)

Elastic Arteries.—Serial tracing of the arteries in the interlobular septums of the fetus revealed that these vessels become the elastic arteries of the adult lung. At birth they have an oval outline in cross section (figs. 1 and 2). The lumens are patent and lined by endothelium applied to a thick, wavy elastic membrane, the internal elastic lamina. External to this is a moderately cellular layer of smooth muscle. In general the muscle fibers are oriented circularly. In the outer portion of the media there are several continuous or discontinuous thin, wavy strands of elastica. External to the latter may be a clear space, which probably represents an artefact caused by fixation. Most externally located is a thick, relatively acellular collagenous adventitia which blends with the peribronchial connective tissue. The lumens of such vessels contain a few erythrocytes. At birth and for a short time thereafter, the width of the lumen is less than or equal to the thickness of the wall.

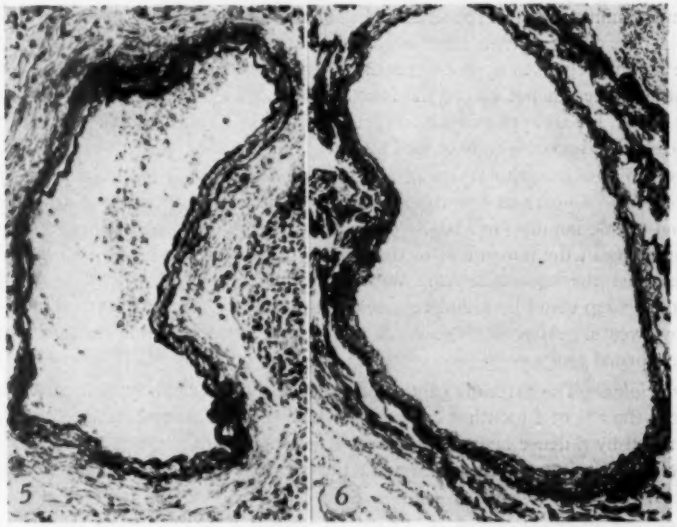

Fig. 5.—An elastic artery of the lung of a child 4 years of age. (Verhoeff's elastic tissue stain counterstained with Van Gieson's connective tissue stain; × 200.)

Fig. 6.—A small elastic artery of the lung of a subject 26 years of age. (Verhoeff's elastic tissue stain counterstained with Van Gieson's connective tissue stain; × 75.)

In the latter part of the first month of postnatal life the luminal diameter of the elastic artery becomes greater than the thickness of its wall. The internal elastic membrane becomes thicker and wavier. The media presents a mixture of collagenous and smooth muscle fibers in its inner two thirds. Its outer third shows several layers of elastic tissue which are thicker than they are at birth. By the age of 6 months these vessels (figs. 3 and 4) have the adult appearance. There is a widely patent lumen, an equal distribution of elastic fibers throughout the media, a thinner adventitia. The luminal outline is oval. By 1 year of age the media is composed predominantly of elastic fibers. At 1 year and later (fig. 5) the change in the vessels is only one of increase in size. After the age of 20 years no progressive increase in size is noted (fig. 6).

Muscular Arteries.—The muscular arteries of the adult evolve from the intralobular arteries of the fetus. In sections from lungs of fetuses, during the last trimester of pregnancy, the lumens of the intralobular arteries are very narrow (fig. 7). The intima is represented by the lining endothelial cells, usually cuboidal in shape. The media is predominantly muscular, and thick. As in the elastic arteries, a clear zone often surrounds the media. The adventitia is a thick layer of collagenous tissue. In the fetus this is as thick as the media. The collagen of the adventitia merges with collagenous strands extending between neighboring alveoli. The cross-sectional outlines of these vessels are circular.

By the time the lobular appearance of the lung is lost, and often shortly after birth, a visible lumen is present in these vessels (fig. 8). During the first six months of postnatal life, the muscular arteries show progressive increase in luminal diameter and external diameter (fig. 9). The walls become relatively and often absolutely thinner than in the fetus and the early newborn. By the age of 6 months the thickness of the walls is noticeably less than the luminal diameter. The internal elastic lamina begins to appear beneath the endothelium usually by the sixth month of postnatal life but not before the fourth month. This membrane becomes a continuous layer. It first appears as interrupted strands of elastica, which eventually fuse. The adventitia becomes thinner and looser.

From 6 months up to 1 year of age the growth in size is the most prominent of features. By 2 years of age the muscular arteries have an internal and often an external elastic lamina (fig. 10). These vessels show gradual thinning of the wall and widening of the lumen and of the external diameter. In all normal lungs from persons past the age of 2 years until about the age of 20 years vessels of the muscular group could be found recapitulating the developmental phases seen in the first two years. After the age of 21 no further developmental changes could be seen in normal lungs.

Arterioles.—The arteriole cannot be identified with certainty at birth or before. At about the age of 1 month it can be identified as a vessel presenting a small lumen surrounded by a dense layer of connective tissue. There is progressive increase in diameter of the lumen, associated with the formation of elastic tissue in the wall (figs. 11, 12 and 13).

The time of development of an elastic lamina in the arteriole varies, but all normal vessels of this class have the layer by the twentieth year postnatally.

In a review of the sections of lungs from adults for comparison with those taken from younger patients it is apparent, as Welch and Kinney[2] have noted, that intimal changes are a noticeable feature in the former. In young subjects normal pulmonary arterial vessels contain little recognizable fibrous tissue between the endothelium and the internal elastic lamina. No pulmonary vessels examined in patients up to the age of 10 years showed any intimal fibrosis. In the second decade an occasional intrapulmonary muscular artery and arteriole shows asymmetric intimal acellular collagenous thickening. In the third decade of life these changes are seen more frequently and include involvement of elastic arteries. Between the ages of 30 and 40 years more of the vessels show asymmetric intimal thickening, while some muscular arteries and arterioles show intimal thickening involving the entire circumference. By the fifth decade more of the intrapulmonary muscular

2. Welch, K. J., and Kinney, T. D.: Am. J. Path. **24**:729, 1948.

arteries and arterioles show symmetric intimal collagenization. The elastic arteries begin to show symmetric intimal fibrosis by this time. In the sixth decade all the changes mentioned are more prominent and most vessels show some degree of fibrous intimal thickening. In general there is an increase in the severity of intimal thickening and in the number of vessels involved as age advances. The changes often appear to be greater in the smaller than in the larger vessels. Except for an occasional vessel in older-aged groups, the intimal fibrosis is never in our experience

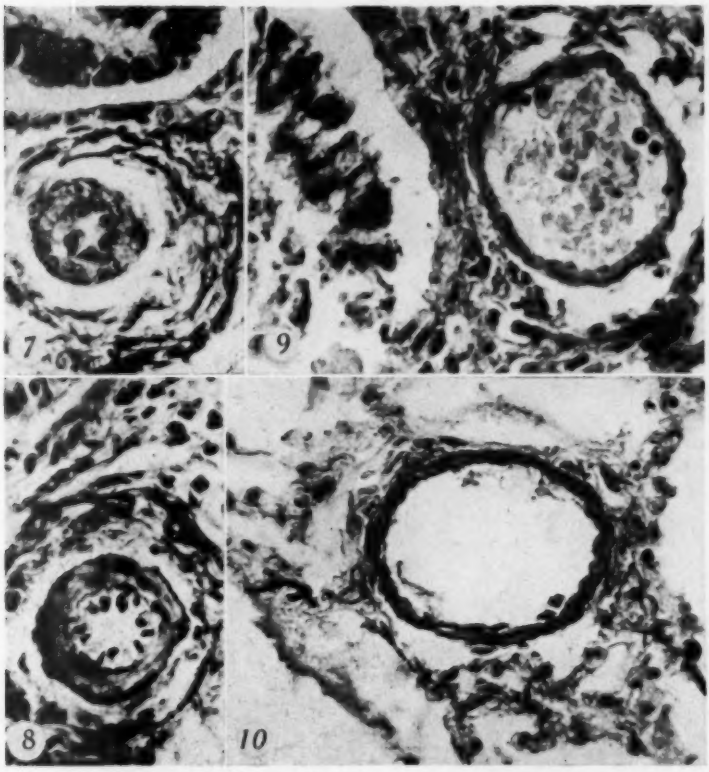

Fig. 7.—A small muscular artery of the lung of a fetus 5 months old. The lumen is narrow. The media is thick. (Verhoeff's elastic tissue stain counterstained with Van Gieson's connective tissue stain; × 450.)

Fig. 8.—A small muscular artery of a lung of an infant 4 days old. The media is still thick. The lumen, though narrow, is readily evident. (Verhoeff's elastic tissue stain counterstained with Van Gieson's connective tissue stain; × 450.)

Fig. 9.—A small muscular artery of a lung of an infant 6 months of age. In contrast to the appearance at earlier ages as illustrated in figures 7 and 8, the lumen is wide and the wall is thin. Irregular strands of elastic tissue represent the early stages of formation of internal and external elastic laminas. (Verhoeff's elastic tissue stain counterstained with Van Gieson's connective tissue stain; × 450.)

Fig. 10.—A small muscular artery of the lung of a subject 17 years of age. The lumen is wide. The wall is thin. The internal and external elastic laminas are well defined. (Verhoeff's elastic tissue stain counterstained with Van Gieson's connective tissue stain; × 450.)

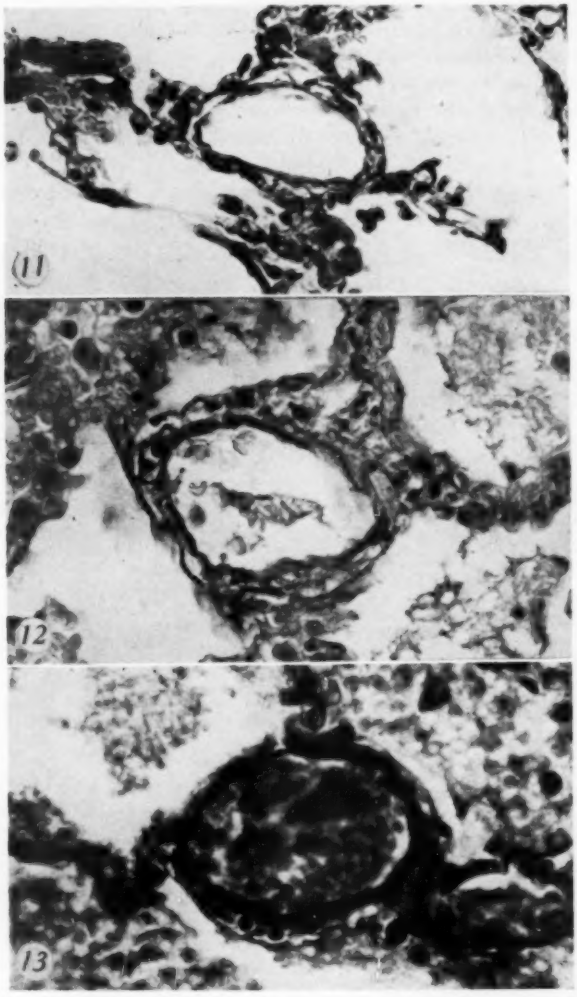

Fig. 11.—A pulmonary arteriole in a subject 9 months of age. The wall is thin. No elastic tissue is identifiable at this stage. (Verhoeff's elastic tissue stain counterstained with Van Gieson's connective tissue stain; × 450.)

Fig. 12.—A pulmonary arteriole in a subject 7 years of age. The wall is thin and contains several strands of elastic tissue. (Verhoeff's elastic tissue stain counterstained with Van Gieson's connective tissue stain; × 435.)

Fig. 13.—A pulmonary arteriole in a subject 26 years of age. The thin wall contains fairly prominent elastic tissue. (Verhoeff's elastic tissue stain counterstained with Van Gieson's connective tissue stain; × 450.)

such as to compromise the lumen to any appreciable extent. It is to be emphasized that as intimal fibrosis makes its appearance the involved vessels would be expected to be less pliable than before these changes become established.

As intimal fibrosis becomes established there is fragmentation of the elastic laminas. In the elastic and muscular arteries the media may show diminution of the elastic and muscle elements and increase of collagenous tissue within the medial layer.

COMMENT

The lungs of the fetus stillborn at term show lobulation. The vessels at the periphery of the lobule are open and filled with blood, while those more centrally situated often have no histologically identifiable lumens. It cannot, however, be assumed that no lumens exist in these vessels in intrauterine life; but it seems justifiable to accept that these vessels have very narrow lumens. Such an arrangement is compatible with a mechanism for maintaining high levels of resistance to pulmonary flow during fetal life.

From the work of Hamilton and associates [3] it is known that in the living fetus the right and left ventricular pressures are about equal. These findings are in accord with those of Barclay and associates,[4] who demonstrated in the living sheep fetus that the right ventricle supplies blood to the descending aorta through the ductus arteriosus, and those of Lind and Wegelius,[5] who demonstrated the same supply in the living human fetus. The high levels of resistance to pulmonary blood flow necessary for this to occur have been explained by citing the dynamics peculiar to the collapsed lung. In this explanation little attention is paid to the possible role of intrinsic mechanisms of the pulmonary vessels for maintaining high levels of resistance to pulmonary blood flow in the fetus. The narrow state of the pulmonary muscular arteries of the fetus may represent an important factor in this phenomenon. That this is so is supported by the fact that in certain cardiovascular malformations there is a high degree of resistance to pulmonary flow after birth when the lungs are fully expanded. In certain of these malformations the structure of the pulmonary muscular arteries is such as to suggest a carry-over from the fetal period.[6] Malformations which exhibit such changes include the Eisenmenger complex,[7] coarctation of the aorta proximal to a patent ductus arteriosus [8] and cor triloculare biatriatum with a wide pulmonary trunk and a narrow subaortic outflow portion of the common ventricle.

The centrilobular pulmonary muscular arteries, shortly after birth, when the lungs are expanding, become dilated so that lumens are easily apparent in them. This may be the anatomic expression of progressive relative fall in the levels of resistance to pulmonary blood flow after birth.

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3. Hamilton, W. F.; Woodbury, R. A., and Woods, E. B.: Am. J. Physiol. **119**:206, 1937.

4. Barclay, A. E.; Franklin, K. J., and Prichard, M. M. L.: The Foetal Circulation and Cardiovascular System, and the Changes That They Undergo at Birth, Oxford, England, Blackwell Scientific Publications, Ltd., 1944.

5. Lind, J., and Wegelius, C.: Pediatrics **4**:391, 1949.

6. Edwards, J. E.: Proc. Inst. Med. Chicago **18**:134, 1950.

7. Civin, W. H., and Edwards, J. E.: Circulation **2**:545, 1950.

8. Edwards, J. E.; Douglas, J. M.; Burchell, H. B., and Christensen, N. A.: Am. Heart J. **38**:205, 1949.

Among the changes in the pulmonary arteries which bring on the appearance of adult vessels are widening of the lumens and thinning of the walls of the vessels by their constituent elements and probably an increase in the number of elements in the walls.

Concomitant with the changes in the wall of the vessels, there is an increase in the luminal diameter. It is the elevation of the ratio of the diameter of the lumen to the thickness of the wall far past unity which is the characteristic feature of the assumption of the intrapulmonary arteries of adult characteristics. Another change in the intrapulmonary arteries and arterioles after birth is the deposition of elastic fibers in their walls. Elastic tissue is consistently present in the elastic arteries at birth, in the muscular arteries by the age of 2 years and in the arterioles by the age of 21 years.

SUMMARY

The intrapulmonary arteries and arterioles of the developing and of the fully developed lungs go through stages which can be traced microscopically from fetal life until the time that the adult appearance and size are reached. In the fetus and at birth the elastic arteries are open, but the ratio of the thickness of the wall to the diameter of the lumen is high, and the adventitia is thick. With growth, the circumference of the wall becomes greater, more elastic fibers are deposited in the media, the ratio of the thickness of the wall to the diameter of the lumen becomes lower and the adventitia becomes thinner. The lumen of the muscular arteries is narrow before birth. Growth of these vessels follows the same course as that of the elastic vessels. In the adult type of muscular artery the media is usually free of elastic tissue except for internal and external elastic laminas. The arterial vessels of the lungs at the age of 6 months show adult characteristics. Before that time the arterioles become recognizable. These gradually show wider lumens, and the walls always remain thin. By the age of 21 years, elastic tissue is found in the walls of the arterioles. In all vessels growth may be by increase in size of existing elements and probably by formation of new elements. Full growth appears to be achieved at about the age of 21 years.

QUANTITATIVE EXFOLIATIVE CYTOLOGY

Differential Counting of Cervical Smears Stained for Glycogen in Cases of Pregnant and Nonpregnant Women

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AND
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THIS COMMUNICATION records an attempt to apply quantitative methods to diagnosis by study of exfoliative cells. In the past seven years several books and scores of articles have appeared based on observations of exfoliative cells. In these the major emphasis has been on qualitative identification of specific cell types, with great attention to cellular structure. Recently various histochemical methods have been developed, and numerous studies of normal and diseased tissues have been carried out. For example, McManus and Findley [1] showed glycogen to be absent from hyperactive epithelium in carcinoma in situ of the cervix and proposed study of the cervical smear by the Schiff periodic acid method. Ayre and Ayre [2] found that cancerous cells in the cervical smears of 10 subjects with squamous carcinoma of the uterine cervix were uniformly lacking in glycogen. These studies suggested that differential counts of vaginal or cervical smears stained to bring out specific intracellular substances might be of assistance in the study or diagnosis of certain physiological and pathological states.

It is reasonable to suppose that exfoliated cells from various portions of the body will reflect the metabolic processes normal and abnormal (including neoplastic) of the tissues from which they are derived. Histochemical technics when applied to exfoliated cells might render information comparable to that obtained from tissue sections. The cells thus stained may be differentially counted, as are blood smears. The presence, absence or degree of the staining reaction for the specific substance brought out by a given histochemical technic may be recorded. In this manner metabolic changes might be quantitatively studied and followed through natural evolution or response to experimental or therapeutic regimens with accuracy and objectivity.

The validity of this assumption was tested in a series of cervical smears made for pregnant and nonpregnant women which were stained for glycogen and differentially counted. Glycogen-containing cells were found in significantly larger

From the Department of Pathology, Emory University School of Medicine, and Grady Memorial Hospital.

1. McManus, J. F. A., and Findley, L.: Surg., Gynec. & Obst. **89**:616, 1949.
2. Ayre, W. B., and Ayre, J. E.: Am. J. Clin. Path. **20**:644, 1950.

numbers among the exfoliated cervical cells from the pregnant women. It has been known for some time that glycogen is increased in certain cells of the female genital tract in pregnancy.[3]

Material for smears was obtained by superficial cervical scraping from 18 pregnant women (the period of gestation ranging from two to seven months) and from 15 nonpregnant women. Six smears were made for each patient and fixed in alcohol-ether. Three of these were digested with saliva before staining as a control procedure, because certain acid mucopolysaccharides, especially mucus, give false positive reactions with glycogen stains. All slides were stained by a modification of the periodic acid routine.[4] In an attempt to enhance the quantitative aspect of the procedure, one each of the digested and undigested slides was exposed to the Schiff reagent for 2, 5 and 10 minutes rather than the 15 minutes indicated by Lillie. It was felt that counting the cells reacting to these varied staining times would afford a more objective determination of the percentages of cells containing varied amounts of the material reacting to the stain than would grading of the intensity of the staining reaction of different cells on a single smear. All counts were done without knowledge of the patient's status.

In all smears the cells staining positively for glycogen were readily differentiated from those failing to take the stain. The number of borderline cells, not clearly positive or negative, was minimal. At least 200 epithelial cells were counted in multiple fields in each smear, including all the cells in any individual field. No attempt was made to determine cell type, nor was attention paid to the details of cell structure.

Six smears, as noted above, were counted in each case, and the number of cells taking (positive) and the number failing to take (negative) the periodic acid stain were recorded. The percentages of positively staining cells in the undigested and the digested smears were computed. A positive staining reaction in an undigested smear indicates the presence of glycogen plus all other substances staining with periodic acid. Since saliva digestion removes only glycogen from the cells, a positive staining reaction in the digested cells indicates the presence of substances other than glycogen. The difference between the percentages of positively staining cells in the undigested and the digested smear should represent the percentage of those cells owing their staining properties to glycogen alone. For convenience these will be termed "glycogen-containing cells." The changes in the percentages of positively staining cells induced by the varied staining times were also recorded. These were found to be noncontributory in this particular series, but it is expected that such values may be of significance in other studies, especially those involving neoplasia. The best differentiation between the pregnant and the nonpregnant women in this study was found in the smears stained for 10 minutes, and only these will be discussed further.

3. (*a*) Mack, H. C.: Am. J. Obst. & Gynec. **45**:403, 1943. (*b*) Papanicolaou, G. N.: Proc. Soc. Exp. Biol. & Med. **67**:247, 1948. (*c*) Wislocki, G. B.; Bunting, H., and Dempsey, E. W., in Engle, E. T.: Menstruation and Its Disorders, Springfield, Ill., Charles C Thomas, Publisher, 1950, p. 23.

4. Lillie, R. D.: Histopathologic Technique, Philadelphia, The Blakiston Company, 1948, p. 145.

In the cervical smears made for 18 pregnant women a mean of 42.0 ± 20.7 per cent of glycogen-containing cells was found. In cervical smears for 15 nonpregnant women a mean of 22.6 ± 15.8 per cent of glycogen-containing cells was found. The significance ratio of the difference between the means of percentages of glycogen-containing cells in the two groups was 2.47. In these smears 14 of 18 pregnant women had more than 35 per cent glycogen-containing cells. If the procedure is judged as a potential pregnancy test, in this instance it had an accuracy of 77.8 per cent. Similarly 12 of 15 nonpregnant women had less than 35 per cent glycogen-containing cells (80 per cent accuracy) in cervical smears.

After digestion, smears for the nonpregnant women showed a mean of 32.3 ± 19.0 per cent of positively staining cells. Smears for the pregnant women showed a mean of 8.6 ± 7.9 per cent of positively staining cells. The significance ratio of the difference between the means of percentages of the positively staining cells in the two groups was 4.53. In this series, therefore, smears for the pregnant women showed a considerably lower percentage of cells containing mucopolysaccharides and additional substances other than glycogen staining positively with the periodic acid routine. In these computations smears for 13 of 15 nonpregnant women had more than 20 per cent positively staining cells (86.7 per cent accuracy). Smears for 16 of 18 pregnant women had less than 20 per cent positively staining cells (88.9 per cent accuracy).

Smears for the pregnant women had a greater percentage of glycogen-containing cells and a lower percentage of cells staining positively after saliva digestion. To give weight to both of these factors, the percentage of the total number of cells staining positively before digestion which owed their staining properties to glycogen was computed. Here the percentage of glycogen-containing cells in the smears was divided by the percentage of positively staining cells in the undigested smears. The nonpregnant women had a mean of 38.9 ± 24.5 per cent of the positively staining cells in their cervical smears owing their staining properties to glycogen. The pregnant women had a mean of 79.6 ± 28.7 per cent in this computation. Fourteen of 15 nonpregnant women had less than 70 per cent of the positively staining cells in their cervical smears owing their staining properties to glycogen (93.3 per cent accuracy). Fifteen of 18 pregnant women had more than 70 per cent of the positively staining cells in their cervical smears owing their staining properties to glycogen (83.3 per cent accuracy). The significance ratio of the difference between the means of the percentages in the two groups was 4.40.

Further studies might determine whether this technic can be developed into a practical pregnancy test. Conditions other than pregnancy, including the menopause and endocrine therapy,[3a] may increase the glycogen content of vaginal smears. Doubtless additional potential sources of error will be uncovered by further experience with the technic.

Smears for three patients with squamous cell carcinoma of the cervix and for one patient with adenoacanthoma of the endometrium were studied by this method. All showed a considerable number of glycogen-containing cells, with some cells staining positively after digestion with saliva. No trend toward a constant variation from the percentages of glycogen-containing or other positively staining cells in the smears for the nonpregnant women could be discerned in these four cases.

This technic may offer a quantitative approach through exfoliative cytology to studies of certain biologic manifestations of physiological and disease states. Differential counts of glycogen-containing cells through various stages or disorders of pregnancy, in tumors, endocrine changes, the menopause and through varied treatment regimens might record objectively many biologic changes not now quantitatively studied.

The application of this technic to other histochemical methods and to exfoliated cells from various sources is in progress.

SUMMARY

A technic of differential counting of exfoliated cells in cervical smears stained for glycogen is described. In a series of 18 pregnant and 15 nonpregnant women, the procedure differentiated the two groups with an accuracy of up to 93.3 per cent, varying with methods of computation. The potentialities of the technic as a research tool and diagnostic test are discussed.

ABERRANT PYLORIC GLANDS IN REGIONAL ILEITIS

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IN A CASE of regional ileitis aberrant glands of pyloric type were observed in the diseased ileum.[1] A search through available literature has not revealed a description of a similar finding in this disease. The atypical glands were then sought in all specimens of large and small intestines examined subsequently and have been found in four of five cases of regional ileitis.

MATERIAL

The number of cases in which autopsy and surgical specimens were examined exceeded 1,000. The cases included ulcerative disease associated with uremia, amyloidosis, gastroenterostomy, tuberculosis, amebiasis, venereal lymphogranuloma, chronic ulcerative colitis, primary and metastatic neoplasms and a variety of unclassified conditions. The aberrant glands were not found in the small bowel in conditions other than regional ileitis and were never found in the colon.

Five cases of regional ileitis were available. In one case aberrant pyloric glands were not encountered. The other 4 cases are the subject of this report. Case 1 was observed at Millard Fillmore Hospital, in Buffalo; cases 2 and 3 and the negative case, at the Veterans Administration Hospital in Temple, Texas; case 4, at the Veterans Administration Hospital in Bronx, N. Y. The cases other than those of regional ileitis were observed at the above hospitals and at the Wasserman Memorial Laboratories of Montgomery County, Amsterdam City Hospital, St. Mary's Hospital and Montgomery Sanatorium, all at Amsterdam, N. Y.

The main clinical features of the four cases in which aberrant pyloric glands occurred are summarized in the accompanying table.

THE LESIONS

Grossly, each of the four specimens presented in the ileum an area of characteristic induration, with thickening of the wall, narrowing of the lumen, and erosion of the mucosa with loss of mucosal folds. The transitions between the grossly normal and the involved areas were abrupt.

Microscopically, in all cases there were extensive erosion and patchy ulceration of the mucosa, a predominantly lymphocytic infiltration of all layers, occasional small abscesses, edema of the submucosa and subserosa, and subserosal lymphangiectasia. The base of the ulcers was formed by fibrinopurulent exudate and patches of nonspecific granulation tissue. Giant cells of foreign body and of

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Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the author are the result of his own study and do not necessarily reflect the opinion or the policy of the Veterans Administration.

1. Liber, A. F.: Unpublished case report read before Buffalo Pathological Society, May, 1942.

Cases of Regional Ileitis with Aberrant Pyloric Glands

Case	Sex	Age	Race	Duration, Yr.	Clinical Findings	Roentgen Report	Previous Operations	Fistula	Specimen		Length of Ileum Involved, Cm.
									Ileum	Colon	
1	F	25	W	3	Reported as typical	Regional ileitis	None	No	100 cm.	Cecum, part of ascending portion	60
2	M	29	N	5	Pain, obstruction, loss of weight	Regional ileitis	Multiple laparotomies	Yes	48 cm.	Appendix	10
3	M	33	W	1	Pain, fever, loss of weight, induration and tenderness in right lower quadrant of abdomen	Consistent with regional ileitis	Ileocolostomy	Multiple recurrent	35 cm.	15 cm.	20
4	M	25	W	9	Pain, diarrhea, vomiting, obstruction, loss of weight, multiple recurrences	Regional ileitis followed by colitis	Appendectomy, ileocolostomy	No	14 cm.	Ileocolostomy site	8

Langhans type occurred in cases 1 and 2. In case 1 they were often surrounded by small groups of epithelioid cells. In case 2 foreign bodies judged to be suture from previous operations were present, but always in uninucleate macrophages, while the giant cells contained no foreign bodies.

Where the mucosa was preserved, the epithelium frequently presented reduction or absence of muciparous goblet cells and the type of metaplasia common to regional ileitis and other chronic inflammatory and ulcerative conditions of the intestine. The nuclei occupied varying levels. They were often hyperchromatic, enlarged and irregular, with occasional mitoses. The cytoplasm stained solidly with eosin or was slightly basophilic. In places the only epithelial structure persisting was the single covering layer of metaplastic cells described by Donchess and Warren.[2]

The glands, whether lined with normal goblet cells or with those showing the common metaplastic changes, were extensively distorted in outline and irregular in caliber. Occasional ramification occurred, with from two to four branches diverging gradually and at acute angles from the main gland and from one another.

The ileum away from the thickened areas, the portions of colon present in cases 1, 3 and 4, and the appendix in case 2 all showed moderate nonspecific infiltration of various types of leukocytes.

THE GLANDS OF PYLORIC TYPE

Type of Cells.—The lining was a single layer of cubic or high rectangular cells with no striated border or other apical differentiation recognizable with ordinary staining methods. The cytoplasm was a uniform, fine, pale pink-staining meshwork containing unstained spaces. Under low power the appearance was that of an extremely pale, nearly unstained cytoplasm. The nucleus was dark, completely basal in position, small and flattened from above downward as if by pressure of the vacuolated cytoplasm apical to it. In some cells, which seemed to be intermediate between the normal goblet cell and the pyloric types, the nuclei, although basal and smaller than those seen in goblet cells, were rounded, or only slightly flattened. But in most areas the transition between the normal and the pyloric epithelium was abrupt, with no intermediate cell types. In all cases observed there were long, uninterrupted stretches of cells having completely flattened and basal nuclei. Mitoses and other evidence of cell division were not seen. The abnormal cells formed a row of remarkable uniformity, with the flattened basal nuclei perfectly alined, giving the cells a distinctive appearance readily recognizable under the lowest microscopic magnifications. The mucicarmine stain was faint or absent in the abnormal intestinal cells, as in the special mucus produced by their prototypes in the pyloric region, while both stained an intense opaque purple with the periodic leukofuchsin method,[2a] as did all varieties of mucus.

Ramification.—The pyloric type of gland seen in regional ileitis ramified in a distinctive manner. The change of direction was abrupt. A vertical tube gave rise abruptly to horizontal branches, which in turn gave off twigs in a plane approximating the vertical. This process was repeated, often many times, with

2. Donchess, J. C., and Warren, S.: Chronic Cicatrizing Enteritis, with Involvement of Cecum and Colon, Arch. Path. **18**:22 (July) 1934.

2a. Lillie, R. D.: Histopathologic Technic, Philadelphia, The Blakiston Company, 1948.

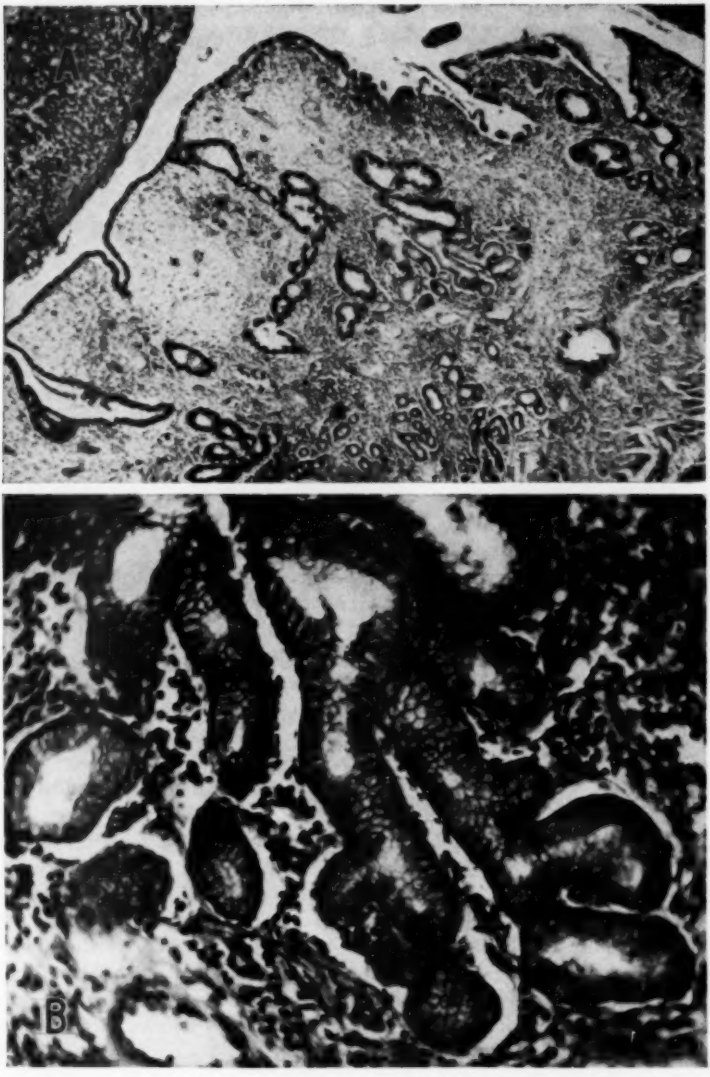

Fig. 1 (case 3).—Ileum. *A*, thickened, eroded, irrugularly nodular mucosa with only rare, distorted, residual glands. The lighter staining glands in the deep layers are of pyloric type. × 32. *B*, detail of the central group of pyloric glands in *A*, showing the characteristic multiple branching with abrupt changes of direction, the regularly alined, flat, basal nuclei and the webby cytoplasm. × 220.

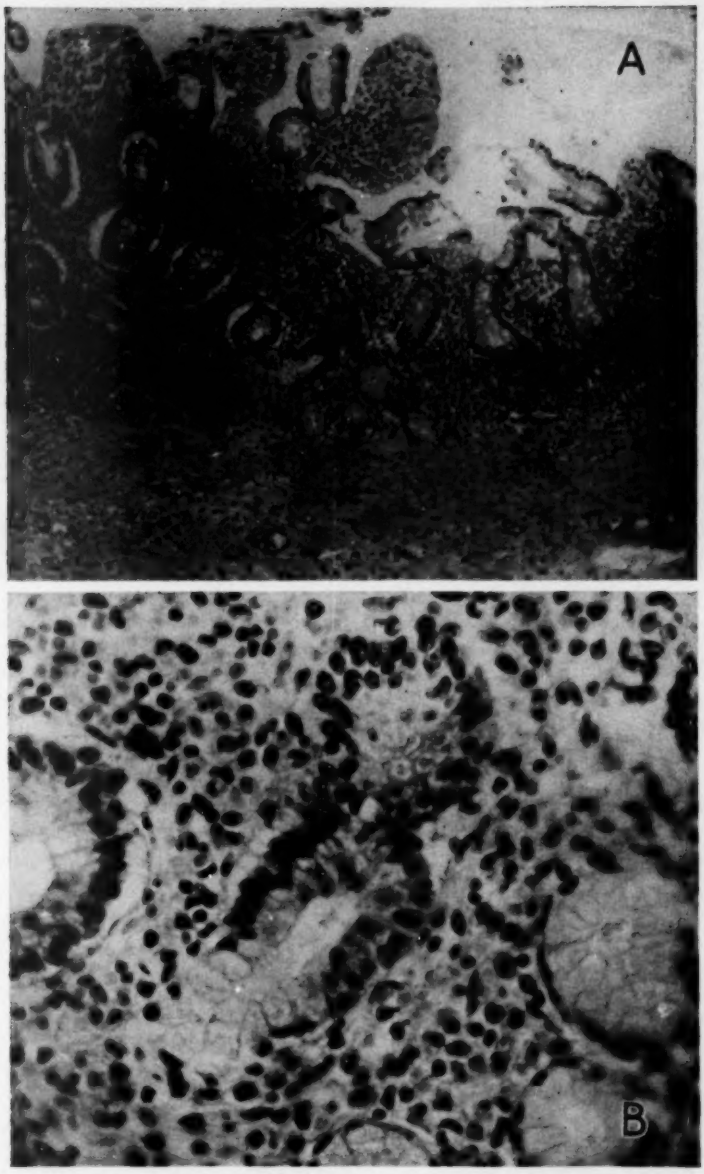

Fig. 2 (case 4).—Ileum. *A*, eroded, thinned area of mucosa. Lymphocytic infiltration of the submucosa. Five sections of pyloric glands lie in the deep mucosa. They are distinguished by pale-staining cytoplasm, flat, basal nuclei and varying planes of section. Two pyloric tubes enter the muscularis mucosae. One pyloric tube is seen as it joins the overlying Lieberkühn crypt. × 100. *B*, detail of *A*, showing the Paneth granular cells at the base of the Lieberkühn crypt, followed downward by the pyloric gland, which soon leaves the plane of section. Parts of three other pyloric tubes are seen at the right and below. × 475.

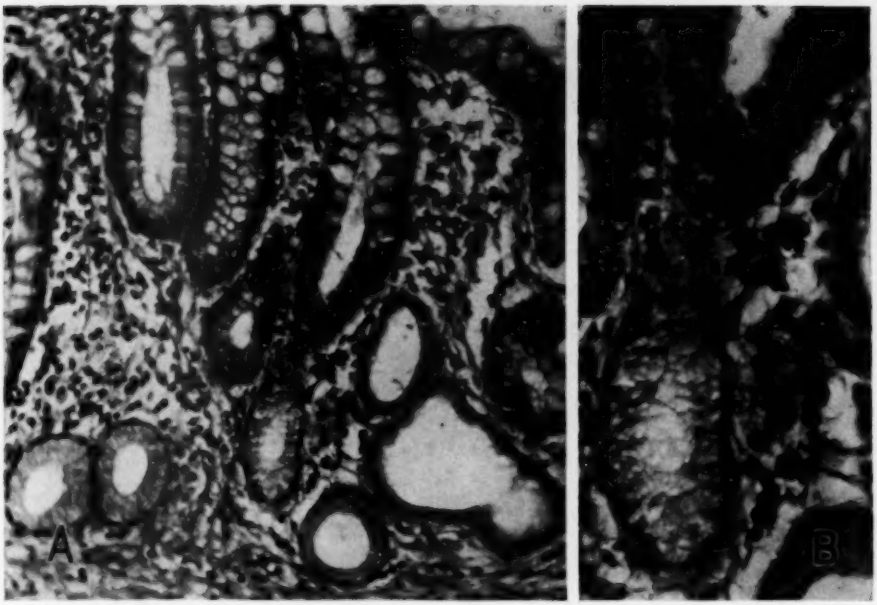

Fig. 3 (case 2).—Ileum. *A*, Lieberkühn crypts above, cut longitudinally, with goblet and Paneth cells; pyloric glands below, most of them cut transversely. × 220. *B*, detail of *A*, showing transition from Lieberkühn crypt to pyloric segment below. Paneth granules are visible above. × 475.

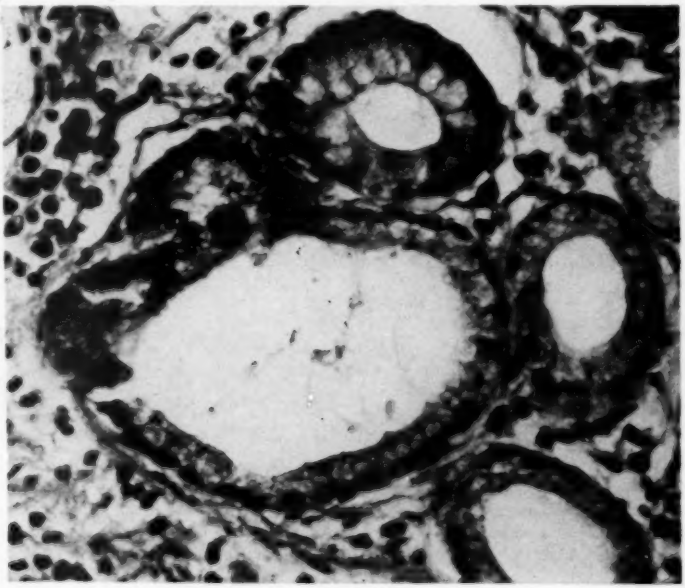

Fig. 4 (case 3).—Ileum. Lieberkühn crypt above, with goblet and Paneth cells, widening abruptly to form the pyloric segment. × 475.

frequently multiple branching at each division, resulting in a large acinous mass extending a considerable distance into the deep layers of the mucosa and sometimes perforating the muscularis mucosae.

Topography Within the Mucosa.—The spatial relations of the pyloric type of tube to the normal crypt were characteristic. The basal Paneth cell segment of the Lieberkühn crypt narrowed sharply and then widened again abruptly to a diameter distinctly greater than that of the overlying goblet cell segment. At the same level the goblet cells and Paneth cells were abruptly replaced by cells of the pyloric type. Occasionally a few Paneth or goblet cells were present among the most superficial pyloric cells, but in all cases they rapidly disappeared. Frequently, there was an abrupt change of direction immediately after the junction of the normal crypt and the pyloric segment. Rarely the pyloric tube penetrated into the submucosa.

Distribution in the Intestine.—Pyloric glands were scattered indifferently over all parts of the intestinal circumference, and at widely separated longitudinal levels, 10 or 20 cm. apart. They occurred both at the edges of the ulcers and in intact but inflamed mucosa. They were never seen in the available segments of colon, uninvolved ileum or appendix.

COMMENT

The atypical glands observed in these four cases of regional ileitis have all the morphological and tinctorial features of the special glands occurring normally in the juxtapyloric regions of the stomach and duodenum: position deep to a histologically different superficial segment, acinous ramification, finely vacuolated cytoplasm and basal flattened nuclei. As in the duodenal Brunner glands, the acinus of the atypical ileitic gland opens superficially into the Paneth cell segment of a Lieberkühn crypt.

The available literature contributes little to the interpretation of these data. The complete review of the pathology of regional ileitis by Warren and Sommers [3] in 1948 contains no reference to the presence of glands of pyloric type, nor was any found in the more recent literature.

In Gould's [4] report of a case of regional enteritis, his figure 14 reveals glands whose cells, contour and ramifications are suggestive of the pyloric type, although these glands are not mentioned in the text. This case appeared typical of regional ileitis but also had features suggestive of coexisting tuberculosis of the intestines, as well as miliary tuberculosis of multiple organs.

In simple or solitary ulcer of the small intestine Robinson and Wise [5] mentioned the presence of "two types of mucosa at the site of the ulcer, which may point to the part played by heterotopic gastric mucosa in the intestinal tract." But, according to Evert, Black and Dockerty,[6] the theory that heterotopic gastric

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4. Gould, S. E.: Case of Regional Enteritis, Am. J. Clin. Path. **17**:955, 1947.

5. Robinson, D. R., and Wise, W. D.: Simple Non-Specific Ulcer of Jejuno-Ileum, Surg., Gynec. & Obst. **70**:1097, 1940.

6. Evert, J. A.; Black, B. M., and Dockerty, M. B.: Primary Non-Specific Ulcers of Small Intestine, Surgery **23**:185, 1948.

mucosa might contribute to the formation of these ulcers could not be substantiated, because gastric tissue had almost never been found in association with them.

In a case of tuberculous ulcer of the colon, Nicholson [7] described glands of gastric type, which appear identical with those reported here. He regarded them as metaplastic.

As an advanced and extensive disease was present in the four cases reported in the present study it is impossible to determine with certainty whether the pyloric atypism was metaplasia secondary to the disease or preexisting heteroplasia. Against heteroplasia is the failure to observe pyloric glands with the same wide distribution in intestines not affected with regional ileitis. The occurrence of pyloric glands scattered over large areas of intestine excludes origin from Meckel's diverticulum or from the localized heterotopic masses of gastric mucosa occasionally observed in the small intestine.[8]

If the aberrant pyloric glands are metaplastic, they pose an intriguing problem in histogenesis. The fundus of the intestinal crypt, to which the aberrant gland is appended and from which it presumably grows, contains in the adult three cell varieties: goblet, Paneth and argentaffin. Can such highly differentiated cells as these be transformed into the pyloric cell with its totally divergent differentiation?

It would be valuable if pieces from all specimens showing regional ileitis were fixed in a manner permitting histochemical enzyme studies which might conceivably help to explain the role of the aberrant glands.

Possibly the additional mucus produced by the large secreting surface of the branched aberrant pyloric glands serves to replace some of the mucus lost by destruction of normal glands and failure of secretion in many residual glands.

SUMMARY

In four of five cases of regional ileitis the affected areas of ileum contained glands of pyloric type, widely scattered. Each tube lined by pyloric epithelium was continuous with and deep to the Paneth cell segment at the base of a Lieberkühn crypt.

The wide distribution of the aberrant pyloric glands excludes their origin from Meckel's diverticulum or other heterotopic tissues.

It is suggested that the pyloric glands are newly formed and that the mucus secreted by them may compensate in part for that lost by the destruction of normal muciparous glands in regional ileitis.

7. Nicholson, G. W.: Heteromorphoses (Metaplasia) of Alimentary Canal, J. Path. & Bacteriol. **26**:399, 1923.

8. Poindecker, H.: Über einen Fall heterotoper Magenschleimhaut in Dünndarm, Centralbl. f. allg. Path. u. path. Anat. **23**:481, 1912. Taylor, A. L.: Epithelial Heterotopias of Alimentary Canal, J. Path. & Bact. **30**:415, 1927. Barták, F.: Heterotopia of Gastric Mucosa in Small Intestine, Časop. lék. cesk. **71**:1070, 1932. Kimpton, A. R., and Crane, A. R.: Heterotopic Gastric Mucosa, New England J. Med. **218**:627, 1938.

VARIANTS OF THE PLATELET THROMBOSIS SYNDROME AND THEIR RELATIONSHIP TO DISSEMINATED LUPUS

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LOS ANGELES

THE FIRST complete description of a disease characterized by acute purpura hemorrhagica, low grade fever, moderate icterus, thrombocytopenia, anemia, terminal cerebral manifestations and rapid, inexorable progression to death, with widespread platelet thromboses of capillaries and precapillary arterioles, was published in 1936 by Baehr, Klemperer and Schifrin.[1] Their study concerned observations made in four young women. Moschcowitz [2] had described a similar case in 1925, but the peculiar hyaline thromboses of small vessels noted by him were not interpreted as platelet thromboses. According to Green and Rosenthal,[3] 18 cases, including three of their own, had been reported by 1949. In none did the picture deviate strikingly from that described by Baehr, Klemperer and Schifrin.[1]

This study is based on two cases in which not only features of platelet thrombosis but features of other conditions were encountered. These observations raised the question whether platelet thrombosis could be related to allergic diseases in general and to lupus erythematosus in particular.

REPORT OF CASES

CASE 1.—H. L., an unmarried white woman, entered the Cedars of Lebanon Hospital for the first time in September 1937, at the age of 43, because of Trichomonas vaginitis. She was successfully treated for this. She complained also of lumbar pain and of swelling of the ankles, the hands and the eyelids, symptoms which were attributed to a psychoneurosis.

Her past history included scarlatina at the age of 9, appendectomy at 16, excision of a uterine tumor at 27, diagnosis of syphilis at 33 and bronchopneumonia at 42. For the syphilis she had been treated intermittently for seven years and finally had been cured. Her family history was irrelevant.

From 1937 on, the patient was under continual observation at this institution for a diversity of complaints. Arthritis, swelling of the legs and the fingers, precordial pain, palpitation, headache, dyspnea, dizziness, malaise, vaginal discharge, folliculitis of the buttocks and furunculosis had required attention. The various diagnoses made included the menopausal syndrome, thrombophlebitis, angioneurotic edema, rheumatoid arthritis and myocardial impairment. Psychoneurosis was considered to be an important factor. For the arthritis diethylstilbestrol was given a five week trial in 1941. Glyceryl trinitrate U. S. P. was prescribed in 1943 for relief of the precordial pain.

From the Department of Pathology, Division of Laboratories, Cedars of Lebanon Hospital.

1. Baehr, G.; Klemperer, P., and Schifrin, A.: Acute Febrile Anemia and Thrombocytopenic Purpura with Diffuse Platelet Thromboses of Capillaries and Arterioles, Tr. A. Am. Physicians **51**:43-58, 1936.

2. Moschcowitz, E.: An Acute Febrile Pleiochromic Anemia with Hyaline Thrombosis of the Terminal Arterioles and Capillaries, Arch. Int. Med. **36**:89-93 (July) 1925.

3. Green, M. A., and Rosenthal, S.: Generalized Blood Platelet Thrombosis: Report of 3 Cases with Necropsy Findings, J. Mt. Sinai Hosp. **16**:110-120 (July-Aug.) 1949.

Dermatologic disorders frequently afflicted this patient. As early as May 1941, when she was 47, erythematous nodules had been observed on the legs. An eruption of discrete nonpruritic papules appeared in June 1942. Xanthoma simplex multiplex involving the knees and the buttocks was diagnosed in December 1943. Early in 1944 specimens of the skin were reported as histologically characteristic of fibroma molle and xanthomatosis of the skin. Later that year new crops of papules and macules appeared.

Operative procedures had included excision of an anal fistula, hemorrhoids and an anal papilloma in October 1942 and removal of two calculi of Wharton's duct late in 1944.

Electrocardiograms were made. That dated 1939 showed no abnormality. In October 1944 and September 1945 left axis deviation was present. Left ventricular preponderance and evidence of myocardial damage also were noted in 1945. Ectopic premature beats were recorded in August 1945.

Roentgenograms of the hands and the wrists in June 1945 showed no abnormality. Those made in April and in June 1946 indicated osteoporosis of the hands, which was considered consistent with atrophic arthritis. A further observation consistent with this diagnosis was the presence of a few small cystic areas at the distal end of the right proximal fifth phalanx in October 1946. In 1946 and 1947 colchicine, cinchophen and antireticular cytotoxic serum were tried for the arthritis—all without avail.

Throughout this 10 year period, 1937 to 1947, blood counts revealed mild anemia, the red blood cell count ranging from 3,830,000 (September 1937 and October 1945) to 4,370,000 (August 1945); the hemoglobin varied from 11.8 Gm. to 13.2 Gm. On June 14, 1946 there was an elevated level of uric acid—6.9 mg. per 100 cc. Subsequent levels of uric acid were normal. All other laboratory observations were normal, including levels of calcium, cholesterol, cholesterol esters and nonprotein nitrogen and dextrose tolerance.

In September 1947 the patient was given sulfadiazine and sulfathiazole, 0.5 Gm. of each, daily, for a suspected infection of the urinary tract. Later that month she had an attack of diarrhea, tenderness in the right upper quadrant of the abdomen and fever. Precordial pain and arthritis were complained of more bitterly than usually at this time. About one week after this episode redness and tenderness appeared over the outer portion of the left leg. Thrombophlebitis was suspected. At this time the red blood cell count was only 3,300,000 and the hemoglobin was 56 per cent. On Oct. 23, 1947 she had an attack of nausea and vomiting, and her temperature was 101 F.; a mild swelling of the left metacarpophalangeal joint was noted, and there was a trace of albumin in the urine. On November 10 an electrocardiogram recorded sinus tachycardia and left axis deviation. The sedimentation rate was 8 mm. per hour, the red blood cell count was 2,200,000, the hemoglobin was 6.7 Gm., and the white blood cell count was 7,100. The following day she complained of a sharp pain in the right side of the chest, but a friction rub was not heard. Nevertheless, possible pleuritis was diagnosed.

Three days later, November 14, the patient was admitted to the hospital for the last time. The history since the previous admittance had included several febrile episodes. Extreme malaise and listlessness were the principal complaints. Her pallor had increased, but evidence of gross hemorrhage was not observed. Scattered rales were heard at the base of the right lung. Her temperature on entry was 102 F., and it continued to reach this height almost daily. The spleen was barely palpable. The albumin-globulin ratio at this time was 3.1 : 3.1. Five hundred cubic centimeters of blood was given four days after admittance. Study of the bone marrow on November 20 revealed moderate pancytopenia. Four days later the spleen, slightly tender, was palpable 1 fingerbreadth below the costal margin. A roentgenogram of the chest on November 20 demonstrated left ventricular enlargement. On November 21 the red blood cell count was 3,200,000, the hemoglobin was 10 Gm., and the white blood cell count was 3,000. On November 26 the excretion of phenolsulfonphthalein was 35 per cent. On December 1 bluish nodules situated over the touch pads of most of the fingers and over the buttocks were diagnosed histologically as chronic nonspecific dermatitis. The thrombocyte count was 28,000, the bleeding time was prolonged to over 25 minutes, and the coagulation time was 10 minutes. The reticulocyte count was 2.6 per cent. On December 4 the red blood cell count was 2,800,000, the hemoglobin was 8.5 Gm., the white blood cell count was 4,000, and the thrombocyte count was 56,000. Study of the sternal marrow on December 10 revealed marked hypoplasia of the erythroid series. At this time the right index finger was painful and there was moderate edema of this digit and of the dorsum of the right hand. The thymol turbidity was 13.4 units.

By December 13 marked deterioration of the patient's general condition had taken place, the pulse rate had risen to 120, and a gallop rhythm was present. Blood counts on December 17 and 22 were low. On December 23 her condition was such that she was unable to retain food and could take only certain forms of liquid. About 500 cc. of blood was administered. Her temperature rose to 104 F. on December 24, her respiratory rate was 28, and she continued to be very weak. Dulness and diffuse rales were heard in the right side of the chest and a few scattered rales in the left side. Bronchopneumonia and pulmonary infarction were diagnosed. Penicillin therapy, 100,000 units every three hours, was begun. On December 25 the pulse rate was 124 and the respiratory rate 40. One thousand cubic centimeters of isotonic solution of sodium chloride was administered that day and again on December 26. Her red blood cell count was 3,360,000, the hemoglobin was 9.8 Gm., and the white blood cell count was 20,000.

On December 27 she had a slight cough, with scanty expectoration of white material. Scattered rales were auscultated, especially at the base of the right lung where the breath sounds were diminished and dulness was present. Scattered squeaks and wheezes also were heard. Digitoxin, 0.2 mg. daily, and streptomycin, 250 mg. every three hours, were added to the therapeutic regimen. Her subjective status was relatively good at this time. A roentgenogram of the chest on December 30 disclosed enlargement of the left side of the heart, chronic passive congestion and a questionable pleural effusion. Five hundred cubic centimeters of blood was given the next day.

On Jan. 4, 1948 the temperature was 101 F. and the pulse rate was 160; she appeared to be in a nearly terminal condition. Blood counts on January 5 were similar to previous counts, and 500 cc. of blood was given one day later. On January 7 the reticulocyte count was 5.1 per cent. She continued on the downhill course and died January 12, at 4:10 a. m. Biopsy of the gastrocnemius muscle on this date demonstrated focal atrophy and an interstitial cellular infiltration.

Throughout the final period of hospitalization urinalyses revealed albumin (1 to 2 plus). Blood nonprotein nitrogen and urea nitrogen and serum cholesterol were all within normal limits.

In summary, this patient with treated syphilis died after an illness which included rheumatoid arthritis, pleomorphic erythemas, angioneurotic edema, thrombophlebitis, anemia, thrombocytopenia and evidences of renal and cardiac damage.

Gross Pathological Observations.—The body was that of a 54 year old white woman, 64 inches (162.5 cm.) in height and weighing approximately 110 pounds (50 Kg.). There were crustaceous lesions of the lips and the face. A fairly well circumscribed violaceous area, measuring 5 by 4 cm., with a large central vesicle was present on the right hip.

Both pleural sacs were completely obliterated by numerous fibrous adhesions binding the lungs to the pleural cage. There were numerous adhesions between the anterior wall of the left ventricle and the pericardium and a few in the region of the aortic knob.

The heart weighed 400 Gm. In the region of the adhesions the epicardium was reddish gray to brown; the largest area of discoloration, measuring 2 by 1 cm., was over the anterior wall of the left ventricle. An area of fibrosis in the lateral wall of the left ventricle measured 2.5 by 2 cm., and, located closer to the base, a focal, sharply circumscribed, yellow-gray area measured 0.5 by 0.75 cm.

There were marked sclerosis and stenosis of the coronary lumens. The aorta was moderately sclerotic.

The right lung weighed 440 Gm. and the left 490 Gm. Numerous bilateral fibrous adhesions formed external sheetlike patterns. The lungs were congested and edematous.

The liver weighed 1,780 Gm. The capsular surface was bound to the diaphragm by numerous fibrous adhesions. On section, the cut surface presented a slightly congested, nutmeg appearance, with focal yellowish infiltrations.

The right kidney weighed 140 Gm. and the left 160 Gm.

The spleen was enlarged, weighing 440 Gm. The capsular surface was slightly nodular. The cut surface was a deep reddish purple, and the slightly enlarged follicles were not as abundant as usual. Many splenic infarcts, the largest being 1 cm. in diameter, were observed.

There were numerous enlarged lymph nodes, most of which were hemorrhagic and had a faint gray cast centrally. Some, particularly those in the abdomen, measured as much as 2.5 cm. in diameter.

The adrenal cortices were tan, and one focal area of hemorrhage was seen in the cortex of the right adrenal.

The lower pole of the right lobe of the thyroid exhibited an area of focal fibrosis.

The brain appeared small and weighed 1,240 Gm. It was markedly edematous, with vascular dilation and engorgement, petechiae, and small flame-shaped hemorrhages. The sulci were enlarged, the gyri thin, and moderate basal arteriosclerosis was noted.

Microscopic Observations.—The heart revealed, in addition to epicardial adhesions, patchy focal necrosis of the cardiac muscle with reactive cellular changes, and occasional cellular infiltration about occluded vessels. A number of small vessels were partially or completely occluded by granular, hyaline "platelet" thrombi and by extrusion of portions of the wall undergoing fibrinoid degeneration.

On examination of the lungs, pleural adhesions and purulent endobronchitis were seen.

In the liver, moderate, focal fatty changes were evident.

In the kidneys, there were focal hyaline necroses in glomeruli with capillary plugging. Scattered patches of distal tubules were involved in a lower nephron type of nephrosis.

The spleen presented many areas of necrosis with thrombosis of the large vessels. Areas suggestive of perifollicular rings of the lupus type were evident in some of the preserved portions.

The lymph nodes showed hemorrhagic dilation of the sinuses.

The bone marrow was hyperplastic.

The adrenal glands were large and hyperplastic.

The thyroid gland revealed a nodular goiter with degeneration.

In skeletal muscle, interstitial fibrosis and proliferation of fixed cellular elements were noted. The muscle fibers were irregularly atrophic.

Pathological Diagnoses.—Platelet thrombosis of the heart; miliary infarcts of the heart; splenomegaly with thrombosis and infarction; glomerulitis; myositis; healed polyserositis.

Case 2.—L. H., a 60 year old white man, entered the Cedars of Lebanon Hospital in May 1948 with a history of fever and chills of two years' duration. This had begun in Chicago, just prior to his moving to Los Angeles. At that time glyceryl trinitrate U. S. P. was being taken for burning precordial pain which was unrelated to exertion but had been diagnosed as angina pectoris. His history included varicose veins and hypertrophic arthritis. There was a family history of asthma of the parents and the siblings.

After his arrival in Los Angeles, two years before admittance to this hospital, the patient suffered intermittent right-sided lumbar pain and complained of weakness and of recurrent chills, fever and sweats. Hepatomegaly and splenomegaly were the principal physical findings. Sixty pounds (27 Kg.) of weight had been lost recently, partially through voluntary dieting, as his previous weight had been 270 pounds (122.5 Kg.).

Physical examination on June 1, 1948 disclosed a ventral hernia, 10 by 12 cm. The pulse rate was 80 and the temperature about 100 F. Laboratory reports in June were as follows: The red blood cell count was 3,940,000; hemoglobin, 9.7 Gm., and sedimentation rate 30 mm. per hour. The platelet count was at first low but rose steadily from 34,720 through 91,820 and 99,870 to 350,280 on June 23; thereafter the platelet count remained normal. Excessive fragility of the red cells was present on June 8 but disappeared 10 days later. At this time the thymol turbidity was 18.2 units, the albumin-globulin ratio was 3.3:3.4, and the cephalin flocculation was 4 plus. The total serum cholesterol was 170 mg. per 100 cc.; the cholesterol esters, 126 mg. per 100 cc. A urinalysis on June 8 showed the urine normal, but thereafter persistent albuminuria and intermittent hematuria were observed, and there were casts and white blood cells in the urine. The blood nonprotein nitrogen level was 42 mg. per 100 cc. and continued to be elevated.

On August 9 the patient complained of pain in the left side, more severe than similar discomfort noted during cystoscopy one week earlier. It radiated from the left lower quadrant of the abdomen to the left side of the chest and to the shoulder. At this time his temperature was 100 F. On August 10 peritoneoscopy and biopsy of the liver revealed slight fatty infiltration of the liver and minimal portal cirrhosis. A congo red test did not indicate amyloidosis at this time. Five hundred cubic centimeters of whole blood were given on August 16. A roentgenogram of the chest on August 21 indicated mild enlargement of the left side of the

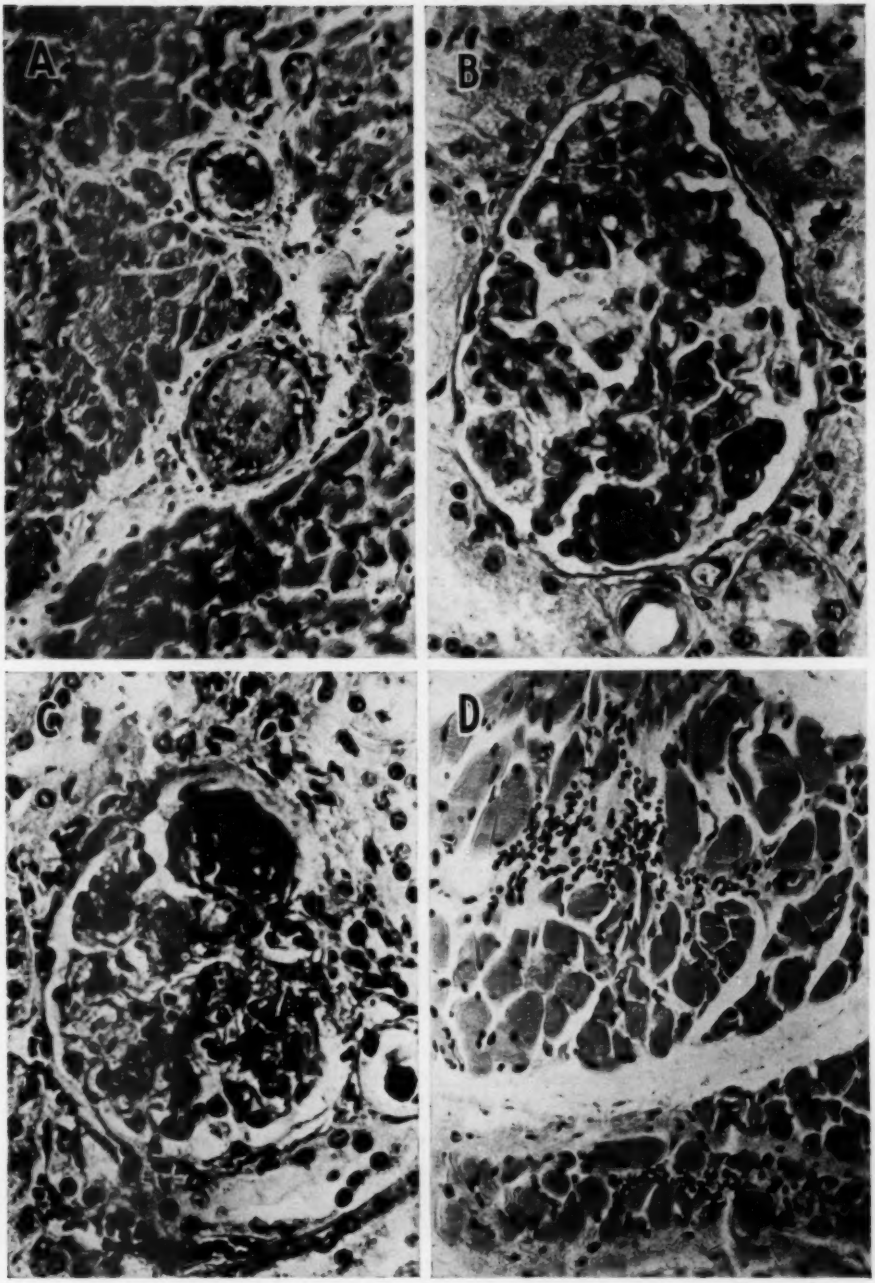

Fig. 1 (case 1).—*A*, thrombi in myocardial vessels; × 120. *B*, thrombi in glomeruli; × 240. *C*, glomerulonecrosis; × 240. *D*, interstitial myositis; × 120.

heart. A biopsy of the skin on September 8 revealed proliferated capillaries containing homogeneous granular eosinophilic thrombi; the diagnosis was "platelet thrombosis." A detailed urinalysis on September 20 was suggestive of a "telescoped sediment," on the basis of which a diagnosis of polyarteritis or lupus erythematosus was deemed likely.

On October 7 the patient was much improved, but the splenic tip became palpable one week later and by October 20 had descended 5 or 6 fingerbreadths below the costal margin. The splenic area was painful.

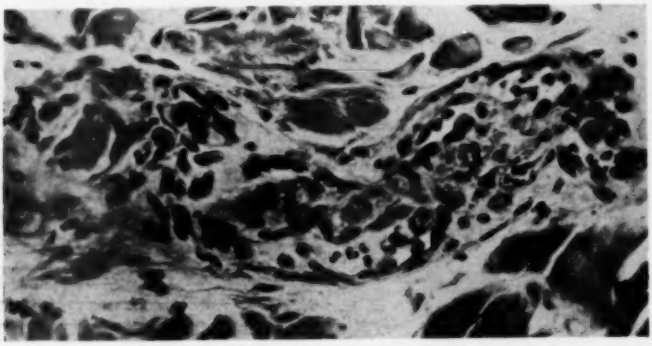

Fig. 2 (case 2).—Thrombi in vessels of the skin; × 210.

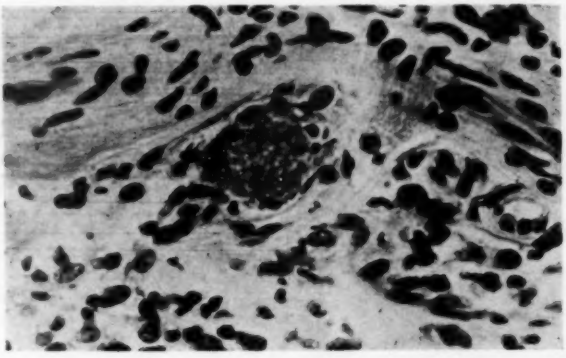

Fig. 3 (case 2).—Thrombosis in skin; × 275.

Frequency and nocturia, ostensibly due to prostatic enlargement, occurred on October 21. Roentgenological examination on November 1 revealed enlargement of the lower pole of the left kidney.

On November 8 and 9 the patient had intermittent pain in the left upper quadrant of the abdomen. Some observers interpreted this to be due to splenic infarction. Walking caused dyspnea and aggravated the pain. On November 15 an electrocardiogram recorded left axis deviation.

The hernial sac was excised on Feb. 15, 1949.

Congestive failure continued and, despite digitalization and the administration of mercurial diuretics, the patient still was in frank congestive failure when discharged March 12. At this time he had a grade 3 apical systolic murmur, cardiac enlargement, hepatomegaly and pitting edema (4 plus).

At the time of his final admittance, March 23, 1949, the patient had severe failure of the left and right sides of the heart, which apparently had been continuous since his discharge from the hospital 11 days earlier. His blood pressure was 195 systolic and 65 diastolic and his pulse rate 120. He had bilateral rales and wheezes, hepatomegaly, dependent edema (2 to 3 plus) and Kussmaul breathing. The venous pressure was 290 mm. of water. Digitoxin, 0.2 mg. daily, and meralluride injection U. S. P. (solution mercuhydrin sodium), 1 cc. intramuscularly three times weekly, were ordered. Albumin (3 plus) was noted in the urine on March 24. The blood nonprotein nitrogen was 72 mg. per 100 cc. and the creatinine 3.1 mg.—its highest value.

On March 25 the respiratory rate rose to 50 to 60. A roentgenogram of the chest disclosed cardiac enlargement, minimal bilateral pleural effusion and pulmonary infarction involving the right middle pulmonary field. The next day the venous pressure was 140 to 160 mm. of water. The temperature continued to be elevated, ranging from 100 to 101.4 F. during this period. The patient's condition continued to deteriorate. Auricular fibrillation appeared, and on March 30 a smooth, lobulated mass about 8 cm. in diameter was noted at the site of the previous umbilical herniorrhaphy. Also, there was severe pain in the right upper quadrant of the abdomen, and a large mass, about 18 cm. in diameter, was observed above and to the left of the umbilicus. At this time the carbon dioxide-combining power was 56 volumes per 100 cc. and the blood chlorides 560 mg. per 100 cc.

By April 2 the patient was in a critical condition with venous distention, fluid and rales in the right side of the chest, tachypnea of 38 to 40, slight cyanosis and a temperature of 103 F.; he was irrational. He died at 2:10 p. m. the following day, April 3, 1949.

In summary, this 60 year old man had precordial, substernal distress, pain in the left flank, fever, transient thrombocytopenia, subcutaneous "platelet" thrombosis, anemia and abnormal urinary findings, which included a "telescopic sediment." Severe, inexorable cardiac failure appeared, and he died despite digitalization, administration of mercurial diuretics and other supportive therapy.

Gross Pathological Observations.—The body was that of a 60 year old white man, 64 inches (162.5 cm.) in height, weighing about 200 pounds (90 Kg.). There was a poorly defined firm mass above and to the left of a transverse supraumbilical scar which measured 15 cm.

Murky tan fluid was found in the pericardial sac, which had many hemorrhagic areas, the largest being 3 cm. in diameter.

The heart was greatly enlarged, weighing 660 Gm. Minimal atherosclerosis of the anterior cusp of the mitral valve was present. Two cusps of the aortic valve had friable, hemorrhagic vegetations on their free edges; the vegetations measured 3 to 5 mm. in diameter.

There was minimal, moderate atherosclerosis of the left anterior descending coronary artery and of the abdominal aorta.

Generalized, diffuse antemortem thrombosis of most of the major veins was observed. Some areas of drainage exhibited venous infarction: The prostatic plexus and the superior mesenteric, splenic, portal and pulmonary veins in particular were involved.

The right lung weighed 840 Gm., and the left, 620 Gm. Arterial and venous thrombosis and dark reddish purple segments of infarction were present bilaterally.

The omentum was plastered up into the left upper quadrant and was firmly adherent to the spleen.

The liver weighed 2,320 Gm. Considerable antemortem clot, involving mainly the portal radicles, was seen in sections through the liver and the porta hepatis.

The left kidney weighed 440 Gm. Its lower pole was displaced by a large retention cyst measuring 9 cm. in diameter and containing clear, colorless fluid. The right kidney weighed 180 Gm. The renal surfaces were finely granular. There was slight thickening of the wall of the bladder, and the vesical mucosa was markedly hemorrhagic.

The enlarged, firm prostate had well defined areas of infarction within some of the prostatic nodules.

The right hernial sac contained omental fat. Numerous hemorrhagic foci were present in both testes.

The 500 Gm. spleen had numerous omental adhesions attached to its capsule. It was markedly congested, the usual structure was obliterated, and numerous infarcts were visible.

In the left adrenal gland, the medulla was especially prominent, and the cortex was moderately hypertrophied. A small typical cortical adenoma was present in this gland.

The brain revealed vascular congestion, focal atheromatosis and some vascular thrombosis. An area of brownish discoloration, 0.2 cm. in diameter, grossly resembling blood pigment, was seen within the left caudate nucleus immediately adjacent to the left ventricle at the level of the anterior limb of the internal capsule.

Microscopic Observations.—The thickened aortic valve contained vascularized fibrohyaline tissue, calcium deposits and superimposed fibrinoid vegetations. Basophilic histiocytes and a sprinkling of miscellaneous inflammatory elements also were noted in the valve. Some superimposed red clot was present on the surface of the aortic valve, and fibrinoid material was deposited on the ventricular surface near the junction of the valve and the myocardium. Pericardial hemorrhage, venous thrombosis and foci of interstitial leukocytes were present.

The lungs contained infarcts and a few scattered thrombi, some showing organization.

Fig. 4 (case 2).—Valvulitis; × 10.

In the intestine, serosal foreign body granulomas were noted focally. Some small vessels contained granular plugs.

In the liver there was irregular necrosis with focal leukocytic reaction and moderate fatty vacuolation. Arterial organized thrombi and central congestion were also noted.

The cyst of the left kidney had a fibrous wall. The kidneys showed diffuse glomerular cellular proliferation and thickening, focal glomerular sclerosis causing rigidity and separation of the glomerular tufts, and an occasional hyaline glomerular ring. An old hyalinized infarct showed tubular coagulation, sclerotic renal vessels and organized thrombi. Focal calcification was present in the medulla.

The bladder was congested. Thrombi, hemorrhage and cellular infiltration, with a few giant cells, were noted in the subepithelial stroma.

The prostate showed foci of necrosis and squamous metaplasia of the glandular epithelium.

Moderate testicular atrophy was observed.

In the spleen, fresh necrosis, focal pooling of blood in the pulp and old infarcts were observed.

The bone marrow was moderately cellular but not particularly hyperplastic.

There was basophilic infiltration of the posterior lobe of the pituitary, and a cluster of hypertrophied pituicytes was noted.

In the adrenals, cortical nodular hyperplasia with lipid-rich cells was observed.

Focal degeneration was present in the brain, with associated collections of vacuolated histiocytes. In one section a focus of vascular proliferation and ectasia involving the subependymal plate was seen.

Pathological Diagnoses.—Cardiac hypertrophy; verrucous aortic valvulitis; disseminated thrombosis, with infarcts of the lung, the brain, the spleen and the prostate; platelet thrombi of the skin; glomerulitis; cyst of the kidney.

COMMENT

The terminal episode in case 1—the fever, the weakness, the progressive anemia, the thrombocytopenia and the rapid death—presented the important characteristics of the platelet thrombosis syndrome. Necropsy revealed many platelet thrombi in the myocardium, which would confirm the clinical impression. However, the focal glomerular necroses and the splenic perifollicular rings suggest a type of tissue change more like that of lupus erythematosus.

The icterus usually associated with generalized platelet thrombosis was not evident, but the abnormal albumin-globulin ratio and the elevated thymol turbidity indicate possible hepatic dysfunction. An alternative explanation would be that a fundamental alteration of the proteins had occurred.

The 10 year history of arthralgia, angina-like attacks, malaise and mild anemia—usually dismissed as a psychoneurosis or the menopausal syndrome—may have been a prodromal smouldering of the disease process, which in this instance was spectacular only in its terminal picture.

In case 2 the chills, the fever and the thrombocytopenia in June 1948 suggested the platelet thrombosis syndrome. This diagnosis seemed to be confirmed by the presence of typical platelet thrombi in a specimen of the skin on Sept. 8, 1948.

Even in 1948 certain features of the disease in this patient were atypical. The two year duration of the illness and the mildness of the nonprogressive anemia were particularly unusual. By Sept. 20, 1948, only 12 days after platelet thrombosis was noted in the cutaneous specimen, the urine contained a "telescopic sediment" suggestive of periarteritis or lupus erythematosus. In April 1949 autopsy revealed verrucous endocarditis and glomerular sclerosis—suggestive of a lupus-erythematosus-like disease.

It appears likely that platelet thromboses constituted the principal early manifestations of the disease and that lupus erythematosus terminated the illness. At one stage the two entities seemed to overlap. In September 1948, when biopsy of the skin disclosed platelet thrombosis, the urinary sediment was typical, if not pathognomonic, of acute disseminated lupus erythematosus, and the platelet count had previously returned to normal.

The abnormal cephalin flocculation and thymol turbidity indicated hepatic dysfunction. Focal hepatic necrosis was found at autopsy and seems to account for these abnormal observations. It is of interest that icterus was absent in this case as well as in case 1, the highest icterus index being recorded as 8.

Pagel [4] recently described two cases of subacute bacterial endocarditis with acronecrotic cutaneous lesions characterized by the presence, in the venous sinuses,

4. Pagel, W.: Acronecrosis Due to Fibrin Thrombi and Endothelial Cell Thrombi, Am. J. M. Sc. **218**:425-431 (Oct.) 1949.

of small thrombi containing fibrin and endothelial cells. Similar platelet thrombi were noted by him in the smaller branches of the pulmonary artery in a third case of subacute bacterial endocarditis. In this case, also, fibrinoid swelling was exhibited in the precapillary arterioles and in the medium-sized arteries of the heart. In two of Pagel's cases there were definite fibrinoid changes in the renal glomeruli, the "wire loop" pattern of lupus erythematosus being exhibited in one. Hyaline intimal infiltrations in the arterioles characterized a rapidly fatal case of purpura. Fibrinoid changes involving the arterial capillaries of the myocardium were present in the fifth case described by Pagel, which was one of fulminant tuberculous septicemia.

Pagel described fibrinoid platelet thrombi and subintimal fibrinoid degeneration as the basic histopathologic changes in these cases. Gore [5] reported similar observations. Subintimal changes occur first in the ground substance and, as the involved area swells, the overlying endothelium ruptures. As the subintimal degeneration progresses, the involved area encroaches on the lumen of the vessel and eventually may break off. The vascular plugs therefore represent, in part, extruded degenerated material.

At this time the preponderant view is that there is not a relationship between the phenomena of platelet thromboses and the so-called collagen diseases, such as periarteritis nodosa, dermatomyositis and lupus erythematosus.[6] However, the cases reported in this paper and two of those described by Pagel [4] indicate that an almost coincidental occurrence of platelet thrombosis and the changes characteristic of "collagen" disease is not uncommon. Atypical verrucous endocarditis, splenic lupus rings, and glomerulitis have been observed [7] in several cases of typical platelet thrombosis, including those reported by Gore.

One may postulate a mechanism that would explain this relationship and which may shed some light on the pathogenesis and natural history of the group of "collagen" diseases to which the platelet thrombosis syndrome might be added. In this scheme the location of the reaction, which probably is hyperergic in nature, is all-important. A reaction occurring in the intima would result in the platelet thrombosis syndrome. A low order of reaction would very likely manifest itself as the localized acronecrotic lesion reported by Pagel. A generalized, fulminant reaction would very likely produce the rapidly fatal syndrome originally described by Baehr and his colleagues.[1] A reaction located within the wall of the vessel would result in a periarteritis nodosa-like entity. Lupus erythematosus would represent such a hyperergic reaction in the perivascular mesenchyme. That this chronologic scheme of tissue involvement may occur is supported by the findings in case 2 and is suggested by the course in case 1.

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7. Friedman, N. B.: The Pathogenesis of Platelet Thrombosis, read before the Seventy-Eighth Annual Session, California Medical Association, Los Angeles, May 8-11, 1949.

What alterations of vascular permeability determine the ultimate location of the sensitization reaction can only be conjectured. Certainly, the fact that lupus erythematosus is predominantly a disease of women suggests that the role of the female steroid hormones may be all-important. That minor alterations of the chemical configuration of such steroids alter completely their effect on the permeability of cellular membranes has been demonstrated recently by Seifter and his colleagues.[8] They found that desoxycorticosterone acted to increase further the permeability of the membranes of the urinary bladder, already rendered abnormally permeable by hyaluronidase, while other steroids, including sex hormones and adrenal corticoids, abolished the increased permeability effected by hyaluronidase.

SUMMARY

Clinical and pathological descriptions of two cases presenting some of the features of the platelet thrombosis syndrome and of lupus erythematosus are reviewed. It is proposed that the platelet thrombosis syndrome be placed in the same category as the so-called collagen diseases, such as periarteritis nodosa, lupus erythematosus and dermatomyositis.

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LIPIDS OF NORMAL AND ATHEROSCLEROTIC AORTAS

A Chemical Study

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THE FIRST to call attention to the fatty nature of the lesions of atherosclerosis was probably Virchow [1] in 1856. Subsequently Gazert [2] and later Baldauf [3] reported that the fat contents of aortas increased with the severity of the arteriosclerosis. Aschoff [4] drew attention to the high concentration of cholesterol in arteriosclerotic aortas, but the first quantitative determinations of cholesterol were performed by Windaus,[5] in 1910. In a systematic investigation Schoenheimer [6] showed that, in addition to cholesterol, atheromatous human aortas contained considerable quantities of phospholipid and some cerebroside. All these lipids increased in concentration with advancing age. Kimmelstiel [7] reported that the concentration of phospholipid and of cerebroside, as well as that of cholesterol, increased with the severity of the disease process. Much of the work in the chemistry of atherosclerosis was reviewed by Wells [8] and by Hirsch and Weinhouse.[9]

There are two distinct factors affecting the lipid composition of the human aorta: (1) the normal process of aging and (2) the pathological process of atherosclerosis. In many of the more recent publications on the lipids of the aorta the effect of age was clearly recognized.[10] Meeker and Jobling [10a] and Weinhouse and Hirsch [10c] minimized the effect of aging by studying intimal tissue that had been separated from the media.

Convenient methods are now available for determining some of the individual phospholipids, notably cephalin, lecithin and sphingomyelin. This has made desir-

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10. (*a*) Meeker, D. R., and Jobling, J. W.: Chemical Studies of Arteriosclerotic Lesions in Human Aorta, Arch. Path. **18**:252 (Aug.) 1934. (*b*) Zeek, P. M.: Am. J. Path. **12**:115, 1936. (*c*) Weinhouse, S., and Hirsch, E. F.: Chemistry of Atherosclerosis: Lipid and Calcium Content of Intima and Media of Aorta With and Without Atherosclerosis, Arch. Path. **29**:31 (Jan.) 1940. (*d*) Faber, M.: Acta med. Scandinav. **125**:418, 1946.

able a further report on the effect of age and of the severity of the disease process on the lipids of the human aorta. The determination of the individual phospholipids has enabled a further distinction to be made between the lipids of aging and those of the disease process. Evidence will also be presented to show that the lipids associated with advancing age are deposited chiefly in the media, while those associated with increasing severity of atherosclerosis are deposited chiefly in the intima.

MATERIALS AND METHODS

Material.—Aortas from 31 male subjects ranging in age from birth to 85 years were examined. The aorta was removed from the body as soon as possible after death, opened and carefully washed. The intima was inspected for signs of atherosclerosis. Depending on the severity of the atherosclerotic lesions, a variable number of pieces of tissue weighing from 1 to 3 Gm. and consisting of the full thickness of the aortic wall were taken. These pieces of tissue were selected to represent various degrees of atherosclerosis seen in the intima. From each aorta one piece of tissue appearing normal on gross examination, and an additional one or two pieces, representing the atherosclerotic lesions, were taken. In all, a total of 59 pieces of aortic tissue was examined. From each specimen a representative block was cut and prepared for histological examination, using hematoxylin and eosin staining.

Analytic Methods.—The adventitia was stripped from each piece of aorta and the remaining intima and media rapidly weighed. No attempt was made to separate the intima from the media, as had been done by some previous workers.[10a, c] This procedure proved difficult and in our hands too unreliable, particularly with normal and severely diseased tissue. The sample was then minced finely with scissors and ground in a mortar. The lipids were extracted successively with 35 cc. portions of ethyl alcohol (once), ethyl alcohol-ether (eight times) and ether (once). Each 35 cc. portion was filtered through an ether-extracted Whatman no. 2 filter paper into a 500 cc. volumetric flask. The tissue remaining in the mortar after these extractions was then washed onto the filter paper with ether and extracted for eight hours with methyl alcohol in a Soxhlet continuous-extraction apparatus. This extract, which contained appreciable quantities of lipid, chiefly phospholipid, was added to the cold alcohol-ether extract and the volume made up to 500 cc. with ethyl alcohol. It is believed that, by using this combination of cold and hot extraction procedures, the extraction of the lipid was complete, with a minimum of oxidation or hydrolysis.

The alcohol-ether extract was then concentrated at 60 C. under reduced pressure and in an atmosphere of nitrogen. The lipids were reextracted into petroleum ether, and the phospholipids were precipitated with acetone and magnesium chloride, as described by Johnson, McNabb and Rossiter.[11] Free and total cholesterol were determined in the acetone-soluble fraction by the method of Schoenheimer and Sperry,[12] incorporating improvements suggested by Sperry and Brand[13] and Sobel and Mayer.[14] Total phospholipid was determined in a sample of the acetone-insoluble fraction as lipid phosphorus × 25 (King[15]). Monoaminophospholipid (phosphoglyceride) was estimated as the total acid-soluble phosphorus liberated from a sample of the acetone fraction after hydrolysis in normal potassium hydroxide for 18 hours at 37 C. (Schmidt, Benotti, Hershman and Thannhauser[16]). Lecithin (phosphatidyl choline) was estimated as the acid-soluble choline in the hydrolysate by the method of Glick[17] and calculated as choline chloride × 5.56 (Hack[18]). Total fatty acid was determined in a sample of the original extract by a

11. Johnson, A. C.; McNabb, A. R., and Rossiter, R. J.: Biochem. J. **43**:573, 1948.
12. Schoenheimer, R., and Sperry, W. M.: J. Biol. Chem. **106**:745, 1934.
13. Sperry, W. M., and Brand, F. C.: J. Biol. Chem. **150**:315, 1943.
14. Sobel, A. E., and Mayer, A. M.: J. Biol. Chem. **157**:255, 1945.
15. King, E. J.: Biochem. J. **26**:292, 1932.
16. Schmidt, G.; Benotti, J.; Hershman, B., and Thannhauser, S. J.: J. Biol. Chem. **166**:505, 1946.
17. Glick, D.: J. Biol. Chem. **156**:643, 1944.
18. Hack, M. H.: J. Biol. Chem. **169**:137, 1947.

modification of the Bloor oxidative method described by Boyd.[19] Details of the analytic procedures and a discussion of their accuracy and specificity are given by Johnson, McNabb and Rossiter.[20]

From these determinations the following calculations were made:

Cholesterol ester = (total cholesterol — free cholesterol) × 1.68.

Sphingomyelin = total phospholipid — monoaminophospholipid.

Cephalin = monoaminophospholipid — lecithin.

Neutral fat = (total fatty acid — ester cholesterol × 0.73 — lecithin × 0.70 — sphingomyelin × 0.44 — cephalin × 0.74) × 1.045.

Total lipid = free cholesterol + cholesterol ester + total phospholipid + neutral fat.

All analyses were done in duplicate. The concentration of lipids is expressed in terms of milligrams of lipid per 100 mg. of fresh tissue, since the dry weight might be expected to vary greatly with the lipid content of the aorta and the fat-free dry weight might be expected to vary with the mineral content.

An attempt was made to measure cerebroside (glycosphingoside) by the method of Brand and Sperry,[21] but because such large amounts of reducing substances were found in the lipid extracts before hydrolytic liberation of galactose, the method gave figures of doubtful reliability. The estimation of cerebroside was therefore discontinued. Any cerebroside present in the aortas would be included in the figures for neutral fat.

Assessment of Degree of Atherosclerosis.—Two methods were used to assess the degree of atherosclerosis:

1. Gross inspection. Each piece of aorta taken for chemical analysis was classified into one of four grades: grade 0 (no evidence of atherosclerosis), grade 1 (discrete plaques not over 2 mm. in diameter), grade 2 (discrete or confluent plaques larger than 2 mm. in diameter but with no evidence of necrosis or ulceration), grade 3 (as in grade 2 but with necrosis and/or ulceration).

2. Measurement of intimal thickness. The thickness of the intima (from the internal elastic lamina to the lumen) was measured in the stained sections with the aid of a micrometer eyepiece. Determined by the method of group correlation, the coefficient of correlation between these two methods of assessing the degree of atherosclerosis was found to be 0.76 ± 0.06.

A third method of assessing the degree of atherosclerosis, not used in the subsequent analysis of the results, was a classification of each piece of aorta into one of four grades according to its microscopic appearance as judged by an independent observer. By the method of group correlation the coefficient of correlation between this method of microscopic grouping and the method of grouping by gross inspection was found to be 0.79 ± 0.06. The coefficient of correlation between the method of microscopic grouping and that of measurement of intimal thickness was 0.84 ± 0.04. It is evident, therefore, that either the gross grading or the measurement of the thickness of the intima gave a reasonable estimate of the degree of atherosclerosis.

RESULTS

1. *Concentrations of the Lipids.*—Table 1 shows the mean concentration of each lipid in the specimens of each grade, arranged according to the severity of the disease. The table also gives the mean thickness of the intima and the mean age for each group. The value of the coefficient of correlation (r) between the degree of atherosclerosis and the concentration of each lipid was obtained by the method of group correlation. The significance of each value of r can be gaged by the value of P given at the foot of each column.

The increase in the mean values for intimal thickness, total lipid, total cholesterol, free cholesterol, cholesterol ester, total phospholipid, lecithin, sphingo-

19. Boyd, E. M.: Am. J. Clin. Path., Tech. Supp. 11, 1938, p. 77.

20. Johnson, A. C.; McNabb, A. R., and Rossiter, R. J.: Biochem. J. **45**:500, 1949; footnote 11.

21. Brand, F. C., and Sperry, W. M.: J. Biol. Chem. **141**:545, 1941.

myelin and neutral fat with each successive grade can be seen. Ester cholesterol expressed as a percentage of total cholesterol increased slightly, while the concentration of cephalin decreased slightly.

The changes reported in table 1 may be the result of one of two processes or both: (1) aging and (2) the pathological process of atherosclerosis, since the

TABLE 1.—*All Aortas—Mean Concentrations of Lipids in the Aortas Classified According to the Degree of Atherosclerosis**

Gross Grade	Mean Age, Yr.	Thickness of Intima, Microns	Total Lipid	Total Cholesterol	Free Cholesterol	Cholesterol Ester	Ester Cholesterol as % of Total Cholesterol	Total Phospholipid	Cephalin	Lecithin	Sphingomyelin	Neutral Fat
0	27	126	1.38	0.31	0.23	0.13	18.36	0.54	0.24	0.15	0.27	0.49
		17	0.15	0.04	0.03	0.03	2.72	0.04	0.01	0.00	0.03	0.09
		(17)	(18)	(19)	(19)	(19)	(19)	(19)	(12)	(12)	(16)	(19)
1	54	352	2.13	0.68	0.39	0.48	40.25	0.60	0.21	0.13	0.26	0.63
		62	0.19	0.07	0.04	0.08	3.06	0.04	0.02	0.01	0.03	0.04
		(11)	(11)	(12)	(12)	(12)	(12)	(11)	(10)	(10)	(11)	(11)
2	64	1,309	5.87	2.37	1.48	1.40	37.59	1.12	0.25	0.28	0.59	1.97
		109	0.66	0.33	0.23	0.24	2.51	0.10	0.03	0.03	0.07	0.25
		(13)	(13)	(14)	(14)	(14)	(14)	(14)	(13)	(13)	(14)	(13)
3	67	2,088	8.51	3.98	2.51	2.47	39.38	1.30	0.15	0.37	0.78	2.40
		378	0.69	0.41	0.36	0.17	2.65	0.10	0.02	0.03	0.08	0.33
		(13)	(13)	(14)	(14)	(14)	(14)	(14)	(14)	(14)	(14)	(12)
r		0.76	0.85	0.86	0.88	0.89	0.53	0.70	−0.28	0.94	0.74	0.74
S.E.r.		±0.06	±0.04	±0.03	±0.03	±0.03	±0.09	±0.07	±0.12	±0.02	±0.06	±0.06
P		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.05	<0.01	<0.01	<0.01

* All concentrations are expressed as mg. per 100 mg. wet tissue ± S.E. mean. The figures in parentheses indicates the number of samples in each group.

TABLE 2.—*Grossly Normal Aortas—Coefficients of Correlation and of Partial Correlation of the Concentrations of Lipids with Age and with Intimal Thickness*

	Coefficient of Correlation with							Coefficient of Partial Correlation	
	Age				Intimal Thickness			Age with Intimal Thickness Excluded	Intimal Thickness with Age Excluded
	N	r	S.E.r	P	r	S.E.r	P		
Total lipid	18	0.88	±0.06	<0.01	0.61	±0.16	<0.01	0.80	−0.02
Total cholesterol	19	0.95	±0.02	<0.01	0.72	±0.12	<0.01	0.90	0.25
Free cholesterol	19	0.95	±0.02	<0.01	0.78	±0.10	<0.01	0.90	0.52
Cholesterol ester	19	0.84	±0.07	<0.01	0.52	±0.18	<0.05	0.78	−0.18
Ester cholesterol as % of total cholesterol	19	0.73	±0.11	<0.01	0.45	±0.20	>0.05	0.65	−0.13
Total phospholipid	19	0.88	±0.05	<0.01	0.74	±0.11	<0.01	0.75	0.40
Cephalin	12	0.01	±0.30	>0.1	−0.17	±0.28	>0.1	0.19	−0.25
Lecithin	12	0.00	±0.30	>0.1	0.53	±0.23	>0.05	−0.61	0.74
Sphingomyelin	16	0.93	±0.04	<0.01	0.63	±0.16	<0.01	0.86	−0.08
Neutral fat	19	0.43	±0.19	>0.05	0.13	±0.25	>0.1	0.48	−0.27

average age of the subjects, as well as the severity of the disease process, in each successive group is greater. What follows is an attempt to disassociate these two processes.

2. *Relation to Age.*—The effect of age on the lipids of the aorta can best be assessed by examining the lipids of those portions of aorta that showed no evidence of disease on gross examination, i. e., the lipids of grade 0 (table 1).

Table 2 shows the coefficient of correlation (r) for the concentration of each of these lipids with age, together with the standard error of r. The high degree of correlation of total lipid, total cholesterol, free cholesterol, cholesterol ester, total phospholipid and sphingomyelin with age is evident. Ester cholesterol as a percentage of total cholesterol and neutral fat are less well correlated with age, while there is no correlation with age for cephalin and lecithin. Table 2 shows that there is a slight degree of correlation between the concentration of some of the lipids and the thickness of the intima, but it was also found that the

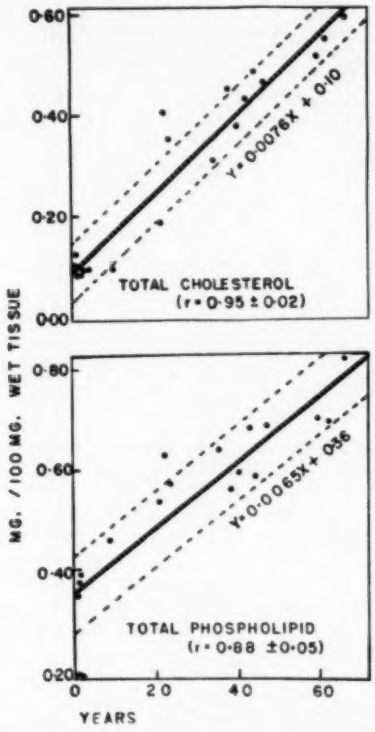

Chart 1.—Grossly normal aortas. Relation of the concentrations of total cholesterol and total phospholipid to age. The solid line gives the calculated regression line and the broken lines the standard error of estimate of the lipid concentration from age.

thickness of the intima and age were significantly correlated (0.70 ± 0.13). Since intimal thickness is an index of the degree of atherosclerosis, it is important to determine if the significant correlation between the concentrations of the various lipids and age is a correlation with age per se or merely a correlation with intimal thickness, which itself is correlated with age. The next-to-last column of table 2 gives the coefficient of partial correlation between the concentration of each lipid and age with intimal thickness excluded, while the last column gives the coefficient of partial correlation between the concentration of each lipid and intimal thick-

ness with age excluded. The coefficients of partial correlation confirm that total lipid, total cholesterol, free cholesterol, cholesterol ester, total phospholipid and sphingomyelin are the lipids most closely correlated with age.

Chart 1 shows the relation of the concentrations of total cholesterol and total phospholipid to age. The calculated regression line for each lipid is shown as the heavy line, while the standard error of estimate of the concentration of each lipid derived from a knowledge of age alone is shown by the dotted lines. Both

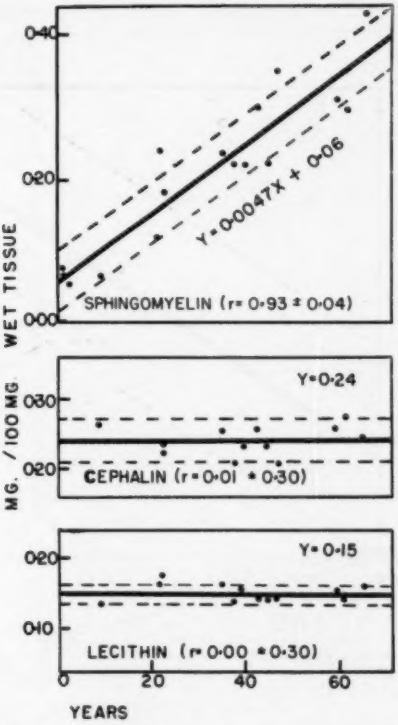

Chart 2.—Grossly normal aortas. Relation of the concentrations of individual phospholipids to age. The solid line gives the calculated regression line and the broken lines the standard error of estimate of the lipid concentration from age.

total cholesterol and total phospholipid increase linearly with age, but total cholesterol slightly more rapidly than total phospholipid.

Chart 2 shows similar data for the individual phospholipids, i. e., lecithin, cephalin and sphingomyelin. It can be seen that sphingomyelin is the only phospholipid that increases with age. The concentration of lecithin and cephalin remains remarkably constant over the span studied.

Table 2 also shows that ester cholesterol as a percentage of total cholesterol is significantly correlated with age. This is brought out in chart 3, which shows

that even in apparently normal aortas a greater percentage of the cholesterol is in the ester form as age increases.

It can thus be concluded that as a result of aging and independently of any obvious disease process there is an increase in the concentrations of total lipid, total cholesterol and total phospholipid. Both free and ester cholesterol increase, but ester cholesterol increases more rapidly than free cholesterol, so that the percentage of cholesterol in the ester form increases significantly. The increase in phospholipid is confined solely to sphingomyelin, no change being observed in the concentration of lecithin or cephalin. There is no change in the concentration of neutral fat.

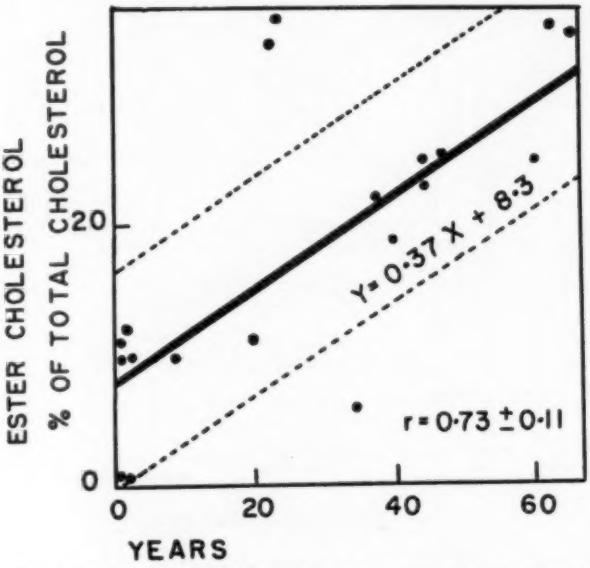

Chart 3.—Grossly normal aortas. Ratio of ester cholesterol to total cholesterol in relation to age. The solid line gives the calculated regression line and the broken lines the standard error of estimate of the value of the ratio from age.

3. *Relation to Atherosclerosis.*—As already seen in table 1, total lipid, total cholesterol, free cholesterol, cholesterol ester, total phospholipid, lecithin, sphingomyelin and neutral fat increase with the severity of the disease as judged by the gross grading. All these lipids, with the exception of lecithin and neutral fat, increase with age. It therefore follows that lecithin and neutral fat increase as a result of the disease process. The same can be assumed for total cholesterol, total phospholipid and sphingomyelin, for the mean concentration of each of these lipids for grade 3 is far greater than the figure that would be expected in an apparently normal aorta as a result of aging alone (charts 1 and 2). Thus, when the gross grading of the aorta is used as a criterion of the severity of the disease, total lipid, total cholesterol, total phospholipid, lecithin, sphingomyelin

and neutral fat may be said to increase with the severity of the disease and quite independently of the age factor.

Chart 4 shows the high degree of linear correlation of total cholesterol and the lesser degree of correlation of total phospholipid with the other index of the degree of atherosclerosis, the thickness of the intima. The degree of intimal hyperplasia is frequently regarded as a useful histological index of the severity of the atherosclerosis. The finding that the degree of intimal hyperplasia is highly correlated with the concentration of total cholesterol ($r = 0.87 \pm 0.03$) indicates

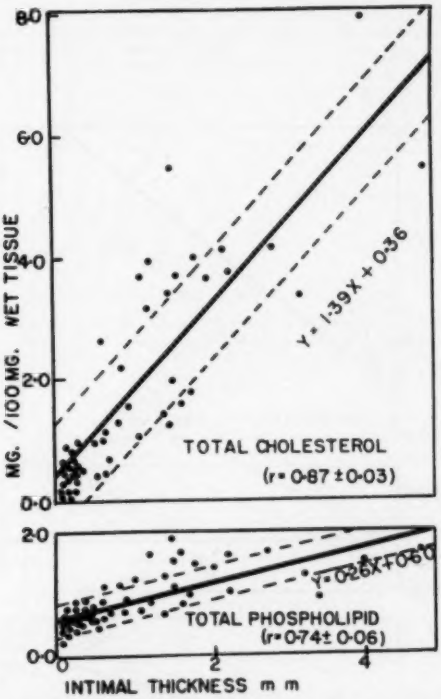

Chart 4.—All aortas. Relation of the concentrations of total cholesterol and total phospholipid to thickness of intima. The solid line gives the calculated regression line and the broken lines the standard error of estimate of the concentration of the lipids from intimal thickness.

that the total cholesterol concentration may serve as a valid chemical index of the degree of atherosclerosis. Evidence for the correctness of this assumption is given in table 3. The coefficients of correlation of the concentration of each individual lipid with intimal thickness on the one hand, and with the concentration of total cholesterol on the other, are very similar. In each instance the lipids that are significantly correlated with intimal thickness, i. e., total lipid, total cholesterol, free cholesterol, cholesterol ester, total phospholipid, lecithin, sphingomyelin and neutral fat, are also significantly correlated with the concentration

TABLE 3.—*All Aortas—Coefficients of Correlation of Lipid Concentrations with Intimal Thickness and with Total Cholesterol Concentration*

	Intimal Thickness				Total Cholesterol Concentration			
	N	r	S.E.r.	P	N	r	S.E.r.	P
Total lipid	50	0.79	±0.05	<0.01	55	0.96	±0.01	<0.01
Total cholesterol	54	0.87	±0.03	<0.01	..	....	.....	.....
Free cholesterol	54	0.83	±0.04	<0.01	59	0.98	±0.01	<0.01
Cholesterol ester	54	0.82	±0.05	<0.01	59	0.91	±0.02	<0.01
Ester cholesterol as % of total cholesterol	54	0.28	±0.13	<0.05	59	0.31	±0.12	<0.02
Total phospholipid	53	0.74	±0.06	<0.01	58	0.85	±0.04	<0.01
Cephalin	46	−0.10	±0.13	>0.1	49	−0.02	±0.14	>0.1
Lecithin	46	0.64	±0.09	<0.01	49	0.80	±0.06	<0.01
Sphingomyelin	51	0.73	±0.07	<0.01	55	0.86	±0.04	<0.01
Neutral fat	51	0.50	±0.11	<0.01	56	0.74	±0.08	<0.01

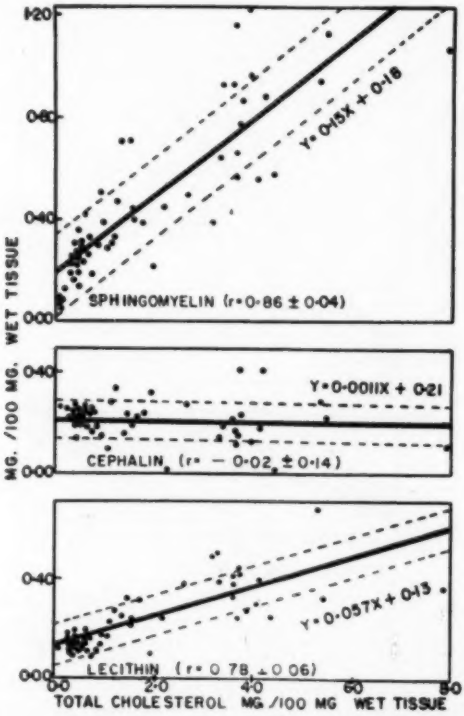

Chart 5.—All aortas. Relation of the concentrations of the individual phospholipids to the concentration of total cholesterol. The solid line gives the calculated regression line and the broken lines the standard error of estimate of the concentration of the individual lipid from the concentration of total cholesterol.

of total cholesterol. The data for the individual phospholipids are plotted in chart 5. Sphingomyelin and lecithin increase with the concentration of total cholesterol, but not cephalin. Thus, whether gross grading, intimal thickness or

cholesterol concentration is used to assess the degree of atherosclerosis, cephalin alone of the phospholipids is not increased.

With increasing severity of atherosclerosis, changes occur in the ratio of free to ester cholesterol (chart 6). Before the appearance of intimal plaques (grade 0) the percentage of total cholesterol that is in the ester form increases sharply as the concentration of total cholesterol increases. As has been pointed out above (chart 3), this is the result of aging in these apparently normal pieces of aorta. However, with the development of intimal plaques (grade 1) the percentage of cholesterol in the ester form still increases, but less rapidly. It is difficult here

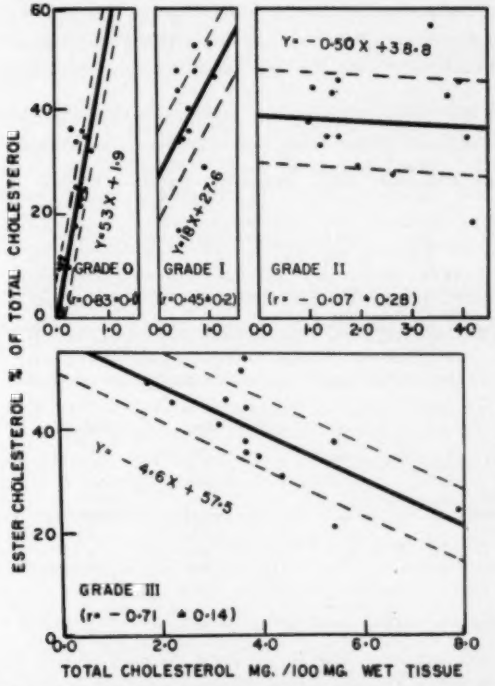
ESTER CHOLESTEROL % OF TOTAL CHOLESTEROL
TOTAL CHOLESTEROL MG. /100 MG. WET TISSUE
GRADE 0
GRADE I
GRADE II
GRADE III
Y= 53 X + 1·9
Y= 18 X + 27·6
Y= - 0·50 X + 38·8
Y= - 4·6 X + 57·5
(r= 0·83 ± 0·0
(r= 0·45 ± 0·2)
(r= - 0·07 ± 0·28)
(r= - 0·71 ± 0·14)

Chart 6.—Ratio of ester cholesterol to total cholesterol in relation to the concentration of total cholesterol. The solid line gives the calculated regression line and the broken lines the standard error of estimate of the value of the ratio from the total cholesterol concentration.

to distinguish between the effect of aging and that of the disease process. In grade 2 the percentage of cholesterol in the ester form no longer increases, and, finally, in the severely affected portions of aorta (grade 3) ester cholesterol increases less rapidly than the free.

Other workers have recognized the difficulty of finding a suitable standard of reference for expressing the changes in lipid concentration with increasing severity of atherosclerosis. In addition to changes in the concentrations of lipids, there are also changes in the amounts of water and nonlipid residue. Table 4

gives the mean concentration of each of the lipids for each grade expressed as a percentage of total lipid. The results are presented in this form in order that they may be readily compared with those of previous workers. With increasing severity of atherosclerosis the total lipid is composed of greater proportions of cholesterol, both free and ester, similar proportions of neutral fat and lesser proportions of phospholipids. The relative amount of cephalin decreases greatly, lecithin less greatly, and sphingomyelin hardly at all. This method of presenting the results suffers from the disadvantage that, because of the rapid increase of cholesterol with rise of the degree of atherosclerosis, some lipids, e. g., lecithin and sphingomyelin, the concentrations of which are actually increasing with the severity of the disease, represent a decreasing percentage of the total lipid.

In order to determine the relation between the concentration of lipid and the degree of atherosclerosis with the age factor entirely eliminated, the difference

TABLE 4.—*All Aortas—Mean Concentrations of Lipids in the Aortas Classified According to the Degrees of Atherosclerosis**

Gross Grade	Total Cholesterol	Free Cholesterol	Cholesterol Ester	Total Phospholipid	Cephalin	Lecithin	Sphingomyelin	Neutral Fat
0	22.5	16.7	9.4	20.1	17.4	10.9	15.2	35.5
1	31.9	18.3	22.5	28.2	9.9	6.1	12.2	29.6
2	40.4	25.2	25.4	19.1	4.3	4.8	10.1	33.6
3	46.8	29.5	29.0	15.3	1.8	4.4	9.2	28.2

* The mean concentration of each lipid is expressed as a percentage of total lipid.

TABLE 5.—*Mean Difference of Lipid Concentration Between Normal or Least Diseased and Most Severely Diseased Portions of Individual Aortas**

	N	Mean	S.E.M.	P	Percentage of Total Lipid
Total lipid	15	5.12	±0.79	<0.01	100.0
Total cholesterol	17	2.68	±0.40	<0.01	50.0
Free cholesterol	17	1.74	±0.33	<0.01	32.5
Cholesterol ester	17	1.37	±0.19	<0.01	25.6
Total phospholipid	17	0.54	±0.10	<0.01	10.1
Cephalin	16	−0.03	±0.28	>0.9	.....
Lecithin	16	0.20	±0.04	<0.01	3.7
Sphingomyelin	16	0.40	±0.07	<0.01	7.5
Neutral fat	15	1.68	±0.36	<0.01	31.3

* All concentrations were found as mg. per 100 mg. wet tissue.

between the concentration of lipid in the most diseased portion of each individual aorta and that in either the normal or the least diseased portion was determined. Table 5 shows the mean of these differences, the standard error of the mean, and the value of *P* obtained in a "t" test to determine whether the means was significantly different from zero. Again it is found that total lipid, total cholesterol, free cholesterol, cholesterol ester, total phospholipid, lecithin, sphingomyelin and neutral fat are significantly increased in the more diseased tissue, while cephalin is not.

COMMENT

Effect of Age.—The finding that the concentrations of total lipid, total cholesterol and total phospholipid increase with increasing age confirms earlier reports.[22]

22. (*a*) Bürger, M.: Ztschr. f. d. ges. Neurol. u. Psychiat. **167**:273, 1939. (*b*) Bjørnsson, J.: Arteriosclerosis: A Chemical and Statistical Study, Copenhagen, Ejnar Munksgaards Forlag,

That cholesterol ester increases more rapidly than free cholesterol agrees with the reports of Schoenheimer,[6] Meeker and Jobling [10a] and Weinhouse and Hirsch.[10c] It is of interest to note that, whereas the concentrations of total cholesterol, total phospholipid and sphingomyelin increase linearly with age, the absolute amount of cholesterol in the whole aorta, as well as the dry weight of the whole aorta, according to Bjørnsson [22b] and Faber,[10d] increases logarithmically.

The finding that sphingomyelin, but neither lecithin nor cephalin, increases with age agrees with the results of Weinhouse and Hirsch.[10c] These workers reported that the increase in the concentration of total phospholipid with age is accounted for by an increase in the ether-insoluble fraction (chiefly sphingomyelin) rather than in the ether-soluble fraction (chiefly lecithin and cephalin). In assessing the effect of age, Weinhouse and Hirsch [10c] did not use aortas that were necessarily free from disease. They separated the intima from the media and analyzed the media only.

In the present study only portions of aorta that appeared normal on gross examination were used. The intima represented a very small fraction of the total bulk of the aorta in these samples. It is considered, therefore, that the lipids laid down in the aorta with increasing age are deposited in the media. Evidence in favor of this view is that in these apparently normal pieces of aorta any correlation between the concentration of lipid and the thickness of the intima was found to be merely the result of a correlation between the small amount of intimal hyperplasia and age (table 2). Additional evidence is that Weinhouse and Hirsch [10c] obtained similar results using medial tissue from which the intima had been separated.

It may thus be concluded that total lipid, total cholesterol and total phospholipid are deposited in the aorta as the result of aging alone. The cholesterol is made up of free cholesterol and cholesterol ester, but the ester cholesterol is deposited more rapidly than the free. The phospholipid is made up entirely of sphingomyelin. These lipids are deposited in the media.

Effect of Atherosclerosis.—In general, the finding of an increase in the concentrations of total lipid, total cholesterol, total phospholipid and neutral fat with an increase in the severity of the disease confirms the results of previous workers.[23] It should be noted that some of these workers determined the concentration of lipid not in the intima plus media but in the intima only.

Windaus [5] and Schoenheimer [6] reported that the concentration of ester cholesterol was relatively greater than that of free cholesterol in atherosclerotic aortas. A contrary view, that the relative proportion of free to ester cholesterol was greater in late lesions than in early lesions, was advanced by Meeker and Jobling.[10a] The results of Zeek,[10b] Weinhouse and Hirsch [10c] and also those reported here support the view of Meeker and Jobling.[10a]

1946. (*c*) Faber, M., and Lund, F.: Human Aorta: Influence of Obesity on Development of Arteriosclerosis in Human Aorta, Arch. Path. **48**:351 (Oct.) 1949. (*d*) Kirk, E. T., and Kvorning, S. A.: Am. Heart J. **38**:476, 1949. (*e*) Schoenheimer.[6] (*f*) Meeker and Jobling.[10a] (*g*) Weinhouse and Hirsch.[10c] (*h*) Faber.[10d]

23 Lehnherr, E. R.: New England J. Med. **208**:1307, 1933. Rosenthal, S. R.: Studies in Atherosclerosis: Chemical, Experimental and Morphologic; Rôles of Cholesterol Metabolism, Blood Pressure and Structure of Aorta; Fat Angle of Aorta (F. A. A.), and Infiltration-Expression Theory of Lipoid Deposit, Arch. Path. **18**:473 (Oct.); 660 (Nov.) 1934. Gazert [2]; Schoenheimer [6]; Kimmelstiel [7]; Meeker and Jobling [10a]; Zeek [10b]; Weinhouse and Hirsch.[10c]

Weinhouse and Hirsch [10c] found that with increasing severity of atherosclerosis the proportion of ether-insoluble phospholipid (chiefly sphingomyelin) in the intima of the aorta increased, while the proportion of ether-soluble phospholipid (chiefly cephalin and lecithin) decreased. An examination of their results shows that in terms of wet weight of intima the concentrations of both fractions increase with the severity of the disease, although the variability of their results is such that the statistical value of these figures is dubious. In the present study it has been found that the increased concentration of total phospholipid is due to a highly significant increase in the concentration of both sphingomyelin and lecithin. The concentration of cephalin, alone, is unrelated to the severity of the disease.

Zeek [10b] reported an increase in the concentration of total fatty acid with increasing severity of disease, a result which has been confirmed here. The "residual fatty acids" have also been found to be increased with the severity of the atherosclerosis. This figure has been called "neutral fat," but it should be remembered that it includes the fatty acids of any cerebroside present and, possibly, some of the sphingosine from cerebrosides and sphingomyelin. The data of McArthur [24] indicate that there is some triglyceride in the phospholipid-free lipid of the intima of atheromatous aortas.

"Atheroma Lipid."—The value determined by the difference between the lipid of the most diseased portion of each individual aorta and that of either the normal or the least diseased portion may be said truly to represent the lipid of atherosclerosis. The concentration of each constitutive lipid is independent of age. The composition of this superadded lipid, or "atheroma lipid," therefore, merits careful study. The relative proportions of free cholesterol and cholesterol ester are quite variable and depend on the severity of the atherosclerosis. If the 17 aortas referred to in table 5 are divided, on the basis of degree of atherosclerosis, into two groups of nine lesser-diseased aortas and eight more severly diseased aortas, the ratio of ester to free cholesterol is 1.4:1 for the former and 0.4:1 for the latter group. The proportion of total phospholipid is fairly constant, averaging a little more than 10 per cent of the total "atheroma lipid." Neutral fat is more variable, but averages 32 per cent of the total. The phospholipid is composed of sphingomyelin and lecithin, and in almost every case more than half of the total phospholipid is sphingomyelin, the average value being 66 per cent. There is no cephalin.

Because the proportions of the individual lipids composing this "atheroma lipid" change with the degree of atherosclerosis, it is illogical to attempt to compare the composition of this lipid with that of serum. However, it may be stated that in the "atheroma lipid" the proportion of free cholesterol is constantly higher, and that of total phospholipid constantly lower, than that usually found in serum lipid. Moreover, the relative concentrations of lecithin and sphingomyelin are almost reversed. According to Hack,[18] whose method of analysis has been used, lecithin constitutes some 80 per cent of the serum phospholipids and sphingomyelin only 15 per cent. Similar figures were reported by Sinclair.[25]

It would appear, therefore, that if the lipids of atherosclerosis are derived from the blood serum, as suggested by Virchow [1] and Aschoff,[4] they must be deposited selectively in the aorta or, alternatively, deposited nonselectively, subsequently to

24. McArthur, C. S.: Biochem. J. **36**:559, 1942.

25 Sinclair, R. G.: J. Biol. Chem. **174**:343 and 355, 1948.

undergo a chemical transformation. The latter view is favored by Hirsch and Weinhouse.[9] But, whatever view is taken, the inescapable fact remains that the "atheroma lipid" of the present study and the considerable quantity of lipid deposited per gram of tissue in the intima of the aorta between the stage of "early fatty plaques" and that of "atheromatous ulcers" in the study of Weinhouse and Hirsch [10c] is not of the same chemical constitution as the lipid of the blood serum. Even if the imbibition theory of Virchow and Aschoff is operative in the early stages of the disease, it certainly cannot explain the changes in the later stage, which are characterized by greater relative amounts of free cholesterol and sphingomyelin.

The evidence is that the lipids deposited in the aorta as a result of the disease process are laid down in the intima. The intima represents an increasing bulk of the aorta with increasing severity of the disease (intimal thickness increases from 126 to 2,088 microns, table 1), and those lipids that increase with the severity of the disease are closely correlated with the thickness of the intima (table 3). It is of interest to note that the composition of the "atheroma lipid" of this study is similar to that which would be obtained if the concentration of lipid in the "normal intima" of the study of Weinhouse and Hirsch [10c] were to be subtracted from that of the stage of "atheromatous ulcers." This provides further evidence for the view that the atheroma lipid is deposited in the intima, since in the study of Weinhouse and Hirsch [10c] intimal tissue only was investigated.

SUMMARY AND CONCLUSIONS

The concentrations of free and total cholesterol, total phospholipid, lecithin and total fatty acid were determined in a series of normal and atherosclerotic aortas. From these figures were calculated the concentrations of cholesterol ester, cephalin, sphingomyelin and neutral fat.

The results were analyzed statistically, especially in relation to age and the degree of atherosclerosis.

As a result of aging, apart from atherosclerosis, there is an increase in the concentrations of total lipid, total cholesterol and total phospholipid. Both free and ester cholesterol increase as a result of aging, but ester cholesterol more rapidly than free. The increase in the concentration of phospholipid is entirely due to an increase in the concentration of sphingomyelin. The relation between the concentrations of these lipids and age is linear. Evidence is presented that the lipids that increase as the result of aging are deposited in the media of the aorta. The concentrations of cephalin, lecithin and neutral fat do not increase with age.

As a result of increase in the severity of atherosclerosis, apart from aging, there is also an increase in the concentrations of total lipid, total cholesterol and total phospholipid. Again, both free and ester cholesterol increase but, with increased severity of the disease process, free cholesterol increases more rapidly than the ester. The increase in the concentration of phospholipid is due to an increase in the concentration of both lecithin and sphingomyelin. The concentration of neutral fat is also increased. Evidence is presented that the lipids that increase as a result of atherosclerosis are deposited in the intima of the aorta. Cephalin, alone of all the lipids studied, appears to play no part in the lipid infiltration of atherosclerosis.

Dr. J. C. Paterson, professor of medical research, University of Western Ontario Faculty of Medicine, gave advice on many aspects of this problem.

The pathological material was largely obtained from the Laboratory Service, Westminster Hospital, Department of Veterans Affairs, London, Ont., Canada.

Case Reports

MELANOMA OF BRAIN AND GALLBLADDER

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IN 1947 Strong,[1] in reporting a case of melanoblastoma primary in the meninges, noted that 36 examples had been recorded previously. MacKay and Hurteau [2] and Conzett and Langford [3] have each added one additional case, bringing the present total of reported cases to 39.

Wieting and Hamdi,[4] in 1907, cited a case in which primary melanoblastoma of the gallbladder had metastasized to the omentum, the pelvic connective tissue and vertebrae. In 1931 Rosenthal [5] reported a primary melanocarcinoma of the gallbladder with metastases in the brain, the lungs, the jejunum and a kidney. Thus only two examples of melanoma taking origin from the gallbladder have been recorded.

This is a report of a case of melanoma involving the brain and the gallbladder, with the original site most likely in the meninges.

HISTORY OF CASE

J. M., a 65 year old married Spanish-American farmer, was admitted to Colorado State Hospital because of indecent exposure and violent threats of four months' duration. The change of behavior along with severe headaches, insomnia, anorexia and loss of weight had been initiated by a "stroke" which resulted in left hemiplegia.

The past history included smallpox in childhood and a traumatic amputation of the right thumb. Alcoholism was noted prior to marriage. A small mass had been present over the right biceps muscle for 10 years. A history of hypertension with edema of the ankles was also obtained.

Physical examination revealed a well developed man of medium complexion, who was partially paralyzed on the left side. A small, nontender, movable nodule was noted in the subcutaneous tissue over the right biceps muscle. The pupils were unequal, with the left one uneven. Both reacted sluggishly to light. The fundi appeared normal. The heart was enlarged. The blood pressure was 130/100 mm. of mercury, and the pulse rate was 112. There was weakness of the left side of the body with spasticity of the left upper and lower extremities. Weakness and flattening of the muscles of the right side of the face were noted. The tongue deviated to the left, and a fine tremor was noted. The deep reflexes were diminished on the

From the Department of Pathology, Colorado State Hospital, Pueblo, Colo.

1. Strong, R. M.: Primary Melanoblastoma of the Cerebellar Leptomeninges with Widespread Extracranial Metastases: Report of a Case, Arch. Path. **44**:477 (Nov.) 1947.
2. MacKay, F. H., and Hurteau, E. F.: J. Nerv. & Ment. Dis. **96**:369, 1942.
3. Conzett, D. C., and Langford, W. R.: J. Iowa M. Soc. **30**:205, 1940.
4. Wieting and Hamdi: Beitr. z. Path. Anat. u. z. allg. Path. **42**:23, 1907.
5. Rosenthal, S. R.: Am. J. Cancer **15**:2288, 1931.

left side but increased on the right. The superficial reflexes were absent on the left side and decreased on the right. There was a slight tremor of the right upper extremity. Tabefaction, slurring of speech and urinary and fecal incontinence were also noted. There was disorientation and emotional flatness together with impaired insight and judgment.

The laboratory findings included an erythrocyte count of 6,200,000 and a hemoglobin content of 19.2 Gm. The volume of packed red blood cells was 55 per cent. The white blood cell count was 12,100, with a normal differential count. The Kahn test of the blood was negative. No urinalysis was recorded. The result of Hanger's flocculation test, the erythrocyte sedimentation rate, the plasma protein levels and the icteric index were within normal range. A chest roentgenogram was not remarkable.

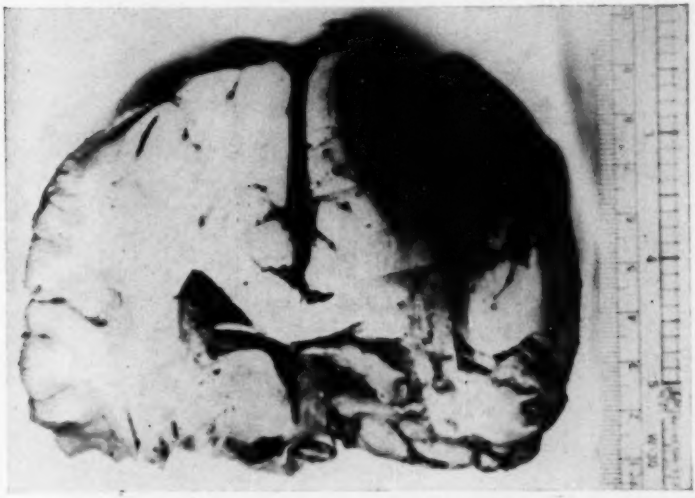

Fig. 1.—Gross section of brain showing a large melanotic tumor involving the right parietal lobe and compressing the surrounding cerebral tissue and lateral ventricle.

During his nineteen days in the hospital the patient remained bedfast. No changes were recorded in the physical or the mental findings. Two hours before death the patient became comatose.

POSTMORTEM FINDINGS

Necropsy was performed on the embalmed body fifteen hours after death. A decubitus ulcer, 1.5 cm. in diameter, was noted over the sacrum. A 1 cm.-sized, discrete, firm, movable, subcutaneous nodule was felt over the right biceps muscle. It did not involve the epidermis, and no pigmentation was present. No other lesions were detected over the tegumentary system. The right thumb was missing. The eyes were

not examined. Internally, there was minimal atherosclerosis of the aorta, and a small Meckel's diverticulum was seen.

The omentum was adherent to a small area of fundus of the gallbladder and stripped away with considerable difficulty, leaving a dark gangrenous-appearing organ, which measured 8 cm. in length and 3 cm. in width and weighed 27 Gm. A large, firm, rubbery, irregular mass was palpated in the fundus of the gallbladder internally. When the gallbladder was opened, about 1 ounce (30 cc.) of viscous, greenish black bile exuded. Attached to the fundus was a brownish black, firm, rubbery, friable mass, which measured 4 by 3 by 3 cm. It had a broad base and had invaded the wall at the point of omental adhesions. Light gray strands of fibrin-like tissue could be seen radiating out into the

Fig. 2.—Gallbladder opened to show a malignant melanoma arising from the fundus. The tumor is sectioned in situ and spread open to show the cut surfaces with diffuse pigmentation throughout.

mass from the base at the gallbladder wall. The tumor projected into the lumen of the gallbladder and had no mucosa over its surface. There was no visible involvement of the liver.

The brain weighed 1,200 Gm. It appeared asymmetrical, with the right side being larger than the left and flattened. All convolutions were smoothed out. From the midportion of the right parieto-occipital region a black, friable mass resembling old hemorrhage protruded from the brain. It measured 4 by 4 cm. on the surface of the cortex. When the brain was sectioned, the tumor was seen to extend into the brain substance to a depth of 3 cm., causing pressure on the surrounding cerebral tissue. The right side of the brain was larger than the left.

The right lateral ventricular system was pushed to the left, with the temporal horn almost obliterated near the tumor. There was also pressure effect with disintegration of slight degree on both the right internal capsule region and the left side of the brain. A small, soft, necrotic area was noted in the pons. There was some congestion of the vessels over the cerebral cortex, but there were no changes in the circle of Willis.

Fig. 3.—Photomicrograph of gallbladder wall showing the melanoma involving chiefly the mucosa and projecting into the lumen. The section is taken from the base of the cancer where adjacent intact mucosa is observed. Hematoxylin and eosin; × 100.

The lymph nodes throughout the body were neither grossly enlarged nor involved with tumor. Likewise, the other organs and tissues were uninvolved.

The lungs showed minimal emphysema, chronic congestion and lipid pneumonia. The spleen showed acute congestion, and the kidneys were slightly hyperemic.

HISTOLOGICAL OBSERVATIONS

Histological examination was performed on specimens which had been fixed in formaldehyde solution. Selected blocks of tissue were embedded in paraffin, sectioned and stained with hematoxylin and eosin.

Sections of the tumor of the gallbladder revealed large, irregular, deeply staining, polyhedral and spindle-shaped cells arranged in an irregular manner, with a slight tendency toward alveolar formation

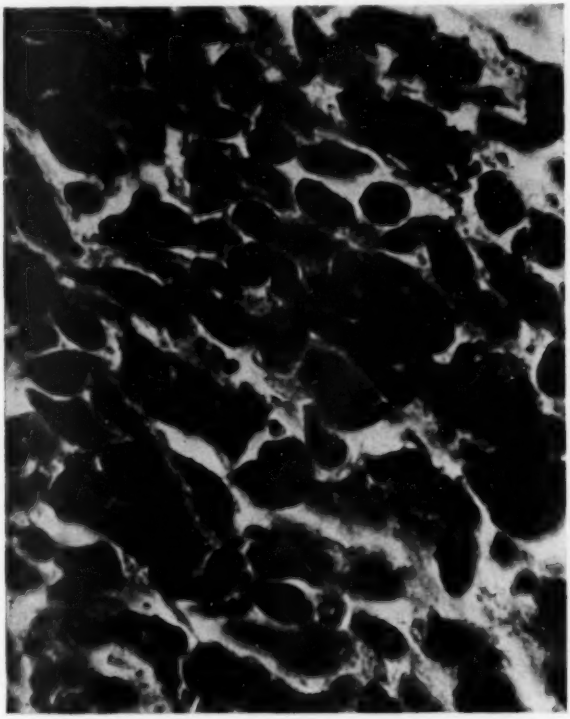

Fig. 4.—Photomicrograph of the neoplasm showing the cellular details and the abundant pigment granules. Hematoxylin and eosin; × 400.

in some areas. Many small black melanin granules were found in the cytoplasm, with the content varying tremendously from cell to cell. These cells penetrated the gallbladder wall in one area, at the point at which the omentum was attached. The individual tumor cells varied tremendously in size and shape. The color ranged from blue to deep brown, depending on the amount of melanin pigment present. The nuclei developed huge sizes, were irregular and deep staining and showed many mitotic figures. A few abnormal mitoses were seen. At the edge

of the neoplasm the cells were necrotic, with considerable debris and hemorrhage, apparently having outgrown their blood supply. There were numerous lymphocytes and large melanin-filled macrophages with very little connective tissue stroma. The fibrous tissue at the base of the tumor was directly connected with that in the gallbladder wall. The gallbladder mucosa was seen to end suddenly at the base of the tumor. The entire picture was typical of melanoma.

The tumor of the brain presented the same arrangement and types of cells as the tumor of the gallbladder. There were numerous pigment-bearing "gitter cells," monocytes, lymphocytes and melanin-filled macrophages. At the edge of the tumor the cancer cells were seen to extend out perivascularly. There appeared to be no supporting stroma, and the blood vessels were not prominent. The surrounding brain tissue showed varying stages of degeneration and necrosis. Several small perivascular hemorrhages were present in the pons.

Sections through one tracheobronchial and two small pericholecystic lymph nodes revealed several foci of large melanin-filled cells. One could not differentiate definitely between macrophages and tumor cells. All other thoracic and abdominal lymph nodes were normal. Stains for iron made on sections of the tumors were negative. Sections of lung, heart, bone marrow, spleen, liver, pancreas, kidney, testis, prostate, thyroid gland, adrenal gland and the remainder of the central nervous system were devoid of melanoma cells.

COMMENT

This patient had two large melanomas, one in the brain and one in the gallbladder. From the gross findings and microscopic observations and from a review of the literature on melanomas no unequivocal answer can be given to the question of which is the primary melanoma.

The general opinion seems to be that melanomas arise from melanoblasts, but there is disagreement concerning their blastomeric origin. Willis [6] stated that "elongated and branched pigment cells are present in the pia mater especially of the base of the brain, especially in dark-skinned people, and are the source of melanotic tumors of the meninges." Many other authors [7] have stated that primary meningeal melanomas occur. We could find no description of melanoblasts occurring in the gallbladder, nor of searches for them. However, of interest are Masson's [8] neuroectodermal theory and Moore's [7d] statement, "If melanoma is derived from cells of the nervous system which migrate out from the neural tube in early embryonic life, it therefore follows that melanoma may be primary in many parts of the body." Maximow and Bloom [9]

6. Willis, R. A.: Pathology of Tumours, St. Louis, C. V. Mosby Company, 1948, p. 899.

7. (*a*) Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940, p. 948. (*b*) Karsner, H. T.: Textbook of Pathology, ed. 6, Philadelphia, J. B. Lippincott Company, 1942, p. 788. (*c*) Boyd, W.: Textbook of Pathology, ed. 4, Philadelphia, Lea & Febiger, 1943, p. 291. (*d*) Moore, R. A.: Textbook of Pathology, Philadelphia, W. B. Saunders Company, 1944, p. 1284.

8. Miner, R. W.: The Biology of Melanomas, New York, New York Academy of Sciences, 1948, p. 39.

9. Maximow, A. A., and Bloom, W.: A Textbook of Histology, ed. 4, Philadelphia, W. B. Saunders Company, 1942, p. 437.

mentioned that little or nothing is known about nerve endings in the gallbladder. Wieting and Hamdi [4] and Rosenthal [5] expressed the belief that squamous cell metaplasia and cancerous transformation account for and make possible primary melanoma of the gallbladder.

The possibility that melanoma may originate simultaneously from both gallbladder and brain or that, in other words, there is a double primary growth must be borne in mind, but one can furnish no data for such a postulation.

If this case is classified as one of primary meningeal melanoma, an entity now accepted by most authors, it fits well into the series of Schnitker and Ayer.[10] They, in reviewing 30 cases of meningeal melanoma, found that two thirds of the tumors had occurred in males, 100 per cent in the white race and that the average age of the patients was 45 years and the average duration of symptoms 7.5 months. Strong [1] mentioned that meningeal melanoma can metastasize to thoracic and abdominal viscera and cited examples in cases of (1) Boit (liver), (2) Ehnmark and Jacobowsky (liver), (3) Foot and Zeek (lungs) and (4) Strong (lung, adrenal gland, stomach). Another questionable case was offered by MacKay and Hurteau (liver, stomach and small intestine). However, our case would either be an exception to or unacceptable to Willis,[6] who stated that in none of the indubitably primary melanotic tumors of the central nervous system (15 cases) have visceral metastases been found. Karsner [7b] mentioned that melanomas of meninges and choroid plexus only occasionally have remote metastases.

If the entity of melanoma of the gallbladder is recognized, a controversy into which we cannot enter, and if the two cases reported as primary melanoma of the gallbladder are accepted as such, this case may be classified as one of primary melanoma of that site. A striking similarity is noted between the case reported by Wieting and Hamdi [4] and our case in both the gross and the microscopic appearance. Our case agrees with that of Rosenthal [5] in that there were lesions of both gallbladder and brain. Both point to an epithelial origin because of the alveolar and glandular arrangement. Our case tended to present such a picture. Willis [6] appeared to be skeptical of the case of Rosenthal,[5] while Ewing [7a] seemed to be doubtful of the case reported by Wieting and Hamdi.[4]

The matter of a route of metastasis could not be definitely settled, but the foci of tumor in the pericholecystic and tracheobronchial lymph nodes indicate that part of the distance between the two organs could have been covered by that route. Since the lungs were not studded with tumor and the foramen ovale was anatomically closed, it follows that the metastasis could not have gone from the lymph to the blood stream via the superior vena cava in the usual manner.

A brief review of the literature on cases of primary melanoma involving organs other than the brain and the gallbladder reveals some interesting statistics. Grace,[11] in 1947, gathered a total of 66 cases of

10. Schnitker, M. T., and Ayer, D.: J. Nerv. & Ment. Dis. **87**:45, 1938.

11. Grace, C. C.: Arch. Otolaryng. **46**:195, 1947.

primary melanoma of the nasal mucosa. His case was the first to be reported in which the patient was a Negro. The tumors, peculiarly enough, always arose from the pigment-free area on the nasal septum or the lateral nasal wall. Knisely and Baggenstoss,[12] in 1946, collected 11 cases of primary melanoma of the adrenal gland and presented one of their own to make a total of 12. Wade,[13] in 1945, accumulated a total of nine cases of melanoma of the small intestine, not including one of his own. Menne,[14] in 1936, published a case of multiple primary melanoma of the small intestine. He mentioned, speaking in relative terms, that melanoma occurs most frequently in the rectum, less so in the small intestine and only occasionally in the gallbladder, the bile ducts and the esophagus. Linder and Wood,[15] in 1936, gathered 79 cases, including one of their own, of melanoma of the rectum. They agreed with Chalier and Bonnet that the origin was from the stratified squamous epithelium of the anal canal. These reports all tend to indicate that this tumor is not as rare in viscera as once thought.

SUMMARY

A case of melanoma involving the brain and the gallbladder is reported. It is suggested that the tumor probably arose in the meninges, but its possible origin in the gallbladder, with metastases in the brain, cannot be expelled.

Medical College of Virginia Hospital.

12. Kniseley, R. M., and Baggenstoss, A. H.: Primary Melanoma of the Adrenal Gland, Arch. Path. **42**:345 (Sept.) 1946.

13. Wade, B. N.: Northwest. Med. **44**:388, 1945.

14. Menne, F. R.: Am. J. Digest. Dis. & Nutrition **3**:786, 1936.

15. Linder, H. H., and Wood, W. Q.: Brit. J. Surg. **24**:65. 1936.

Notes and News

Appointment.—Dr. Russell Landram Haden, medical educator and recently head of the department of medicine at the Cleveland Clinic, has been appointed medical director of the Red Cross National Blood Program. Dr. Haden succeeds Dr. Louis K. Diamond, Boston hematologist and a member of the Harvard Medical School faculty, who resigned to return to his duties at Harvard and at Children's Hospital.

Laboratory Training Courses.—A schedule of the laboratory training courses offered by the Communicable Disease Center during 1951 may be obtained from Officer in Charge, Laboratory Training Services, Communicable Disease Center, United States Public Health Service, P. O. Box 185, Chamblee, Ga.

Meeting of American Academy of Forensic Sciences.—The American Academy of Forensic Sciences will hold its third annual meeting March 1, 2 and 3, 1951, at the Drake Hotel, Chicago. All persons desiring to present papers are requested to contact Dr. A. W. Freireich, chairman of the program committee, 180 Hempstead Avenue, Malverne, N. Y.

SPECIAL NOTICE

For the general information of indexers, catalogers and library personnel with reference to the listing of the new title of this journal, we advise that the *Quarterly Cumulative Index Medicus* policy be followed. The *Quarterly Cumulative Index Medicus* with its volume 49 (January-June 1951) will list this journal under the new title, so that the letters A. M. A. will be included as an integral part of the title. For the completion of volume 48 (July-December 1950) the *Quarterly Cumulative Index Medicus* will continue to use the old title, without adding the designation A. M. A.

Books Received

STEROID HORMONES AND TUMORS. TUMORIGENIC AND ANTITUMORIGENIC ACTIONS OF STEROID HORMONES AND THE STEROID HOMEOSTASIS: EXPERIMENTAL ASPECTS. By Alexander Lipschutz, M.D., formerly professor of physiology, honorary member of the Royal Society of Medicine (endocrinology), recipient of the Charles M. Mayer Cancer Award of the National Science Fund of the National Academy of Sciences, 1944, and director of the department of experimental medicine of the National Health Service of Chile. Pp. 309, with 111 illustrations. Price $6. Williams & Wilkins Company, Mt. Royal and Guilford Avenues, Baltimore 2, 1950.

This book is divided into three main parts, dealing with the tumor-forming action of steroids, with their anti-tumor-forming properties and with the streoid balance and the antitumoral autodefense of the body. The author uses new words, such as "tumorigenesis," "tumorigenic" and "antitumorigenic," referring to the origin and prevention or therapy of all kinds of neoplasms, not only cancers, but also benign tumors. These words are also used to denote localized hyperplasia and not necessarily true neoplastic growth. This is particularly pertinent because throughout the text one sometimes encounters difficulties in deciding whether what is produced experimentally is a true tumor or a localized area of hyperplasia or "insular hyperplasia" or "insular metaplasia," as it is called by the author. Thus, a reproduction of a fibroadenoma of the mammary gland resembles hyperplasia of the breast much more than a true tumor.

This monograph is the result of a 15 year period of work, part of which has been presented in communications (theses) of the University of Chile. Throughout the text an attempt is made to show that all estrogens, with any exception, natural or artificial, free or esterified, can become toxic for the body and tumorigenic. It is stressed that it is not possible to apply to man experimental results obtained on laboratory animals relating to steroids and tumors. In regard to both spontaneous and experimental neoplasms it is maintained that there is a succession of evolutional phases: those of dependent and those of seemingly autonomous growth. In the phase of dependent growth atypical proliferation and differentiation is going on only in the presence of the stimulus which has induced neoplastic possibilities. However, in the phase of autonomous growth proliferation is going on in the absence of this stimulus. The main theme of the book revolves about the problem of whether steroids may interfere in the dynamics of cancer and about the quantitative and the timing conditions under which estrogens become able to induce atypical growth.

The author stresses that the object of this book is to give a summary of experimental work and not to discuss details related to steroid treatment of tumors. However, it is emphasized that it seems contrary to nature to administer estrogens uninterruptedly and that it is contrary to existing knowledge to administer estrogens by implanting pellets, or tablets, beneath the skin. Hormones should be administered by mouth for about two weeks, starting again after an interval of two weeks and so on. A deviation from the rule of the rhythm seems admissible only in the treatment of mammary cancer in aged women or in the treatment of prostatic carcinoma in men.

The book presents a fountain of knowledge and of quotations from the literature with reference to a very timely subject. It has excellent illustrations and valuable and useful sketches and tables.

PNEUMOCONIOSIS. Arthur J. Vorwald, M.D., director of the Trudeau Foundation and the Saranac Laboratory, editor. With the collaboration of Manfred Bowditch, A.B., Thomas M. Durkan, M.E., and Theodore C. Waters, LL.B. Pp. 659, with 196 illustrations and 79 tables. Price $7.50. Paul B. Hoeber, Inc., Medical Book Department of Harper and Brothers, 49 E. 33rd St., New York 16, 1950.

This symposium on pneumoconiosis with emphasis on beryllium and bauxite fumes and a discussion on compensation for occupational diseases was collected and dedicated as a memorial volume to Leroy U. Gardner. Dr. Gardner, a former director of the Trudeau Foundation, was

one of the pioneers in the experimental investigation of the effects of dust on living tissue. It was during the years of World War II that a new disease appeared which was caused by the inhalation of dust containing beryllium, recognized in certain industrial employees. About the same period another new disease occurred among workers exposed to bauxite fumes and was described as Shaver's disease.

This volume consists of seven sections. Part I treats of the industrial aspects of beryllium; part II, the clinical phases of acute beryllium poisoning; part III, the roentgenological physiological and pathological aspects, with a chapter on the postmortem observations in cases in which the patients had been exposed to beryllium; part IV, biochemical, toxicological and experimental studies with beryllium and a chapter on the connection of these studies with atomic energy production; part V, the experimental aspects of the beryllium problem; part VI, Shaver's disease (the clinical and chemical aspects), and part VIII, compensation for occupational disease, with discussions pertaining to the viewpoints of workmen's compensation administration.

The section on the pathological aspects of beryllium poisoning reviews the analogy of this disease with pneumonoconiosis, and there is a discussion of the gross and microscopic changes. The pathological features of this disease are analyzed and compared with those of Boeck's sarcoid, silicosis and tuberculosis. In view of the fact that beryllium poisoning comes under the head of an industrial disease, the cases are usually referred to the coroner's or the medical examiner's office for autopsy. Hence, the pathologist should be familiar with the pathological aspects of this disease. There is also a discussion on the pathology of acute experimental beryllium poisoning.

Shaver's disease occurs among workers who are engaged in the processing of bauxite for the manufacture of corundum. This disease sometimes follows the inhalation of the fumes and is accompanied by fibrosis of the pulmonary tissue. Spontaneous pneumothorax is a special feature observed at autopsies performed in the fatal cases. The other pathological findings include pigmentation of the lung, irregular areas of fibrosis, subpleural emphysema and thickening of the pleura. In the experimental studies using stack fumes of bauxite, the animals developed a fibrous lung condition showing characteristics not unlike those exhibited by the human cases.

The Leroy U. Gardner Memorial Volume on Pneumoconiosis, with emphasis on the subjects of beryllium, bauxite fumes and compensation, is recommended to all physicians. The pathological discussion of these clinical entities should be exceedingly valuable to all pathologists.

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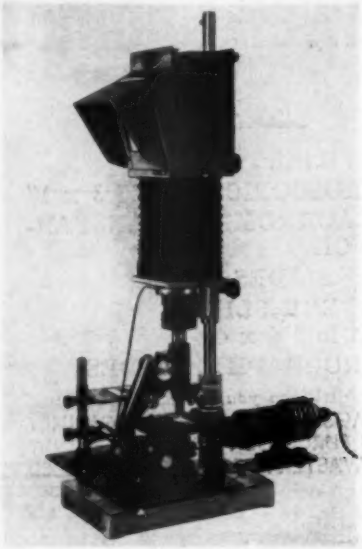

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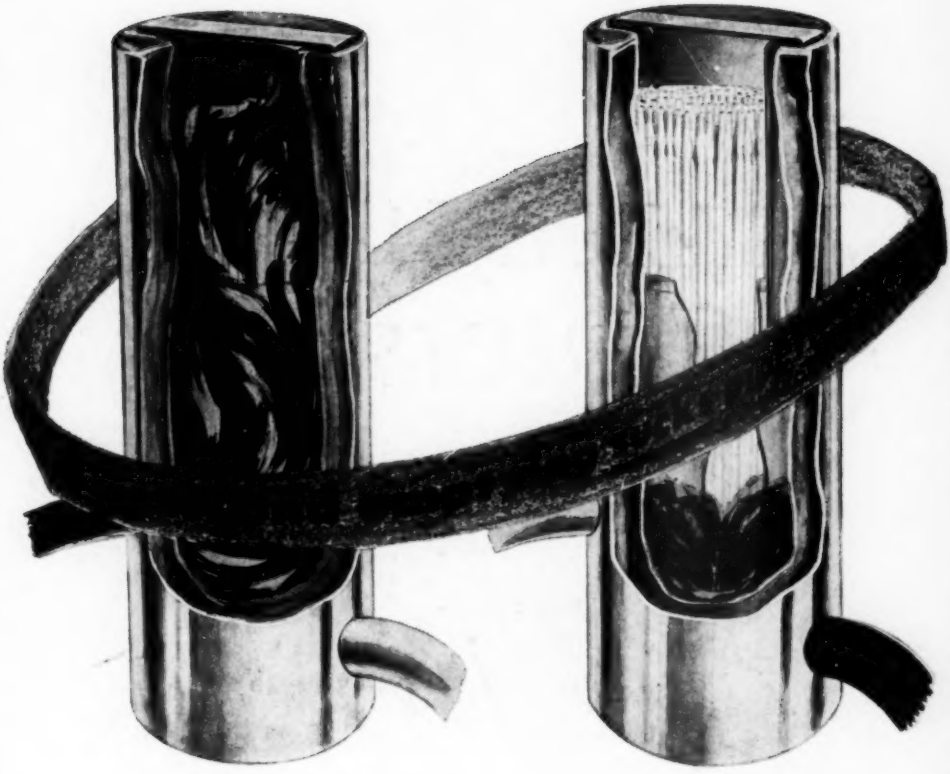

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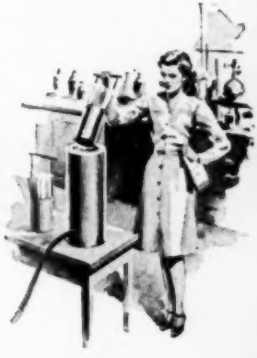

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